Ontogenic Resistance in Grapevine Leaves to Powdery Mildew

by

Angela Maree Merry

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DECLARATIONS

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Angela Maree Merry

ABSTRACT

Erysiphe necator causes powdery mildew in species of *Vitaceae*, including the widely cultivated grapevine *Vitis vinifera*. This obligate biotroph colonises green, juvenile tissues, which if not protected by fungicides can lead to loss of grape yield and wine quality in many viticultural regions worldwide. The extent of pathogen colonisation varies according to leaf maturity and the term ontogenic resistance has been used to describe leaves that become less susceptible to infection by *E. necator* as they age.

The effect of environment during shoot growth on the expression of ontogenic resistance was examined by exposing developing shoots of Cabernet sauvignon in the glasshouse to temperatures set at 18°C or 25°C prior to inoculation with *E. necator* conidia. Powdery mildew severity on leaves, after incubation for 14 days at 25 ± 5°C, initially increased and then decreased as leaves matured beyond a lamina length of 30 mm (leaf position 1) for shoots with an average rate of leaf emergence of 0.54 leaves per day (25^{o}C glasshouse) or 0.23 leaves per day (18^{o}C glasshouse) prior to inoculation. The higher rate of leaf emergence resulted in a greater proportion of diseased leaves per shoot and a higher disease severity for the modal leaf position expressing maximum severity, which was position 4.4 for plants previously exposed to 25^{o}C and position 3.7 for shoots developing at 18^{o}C. Position 4.4 was similar to the mean modal leaf position of 4.2 for the maximum percentage of conidia that formed secondary hyphae for shoots developing at 25^{o}C. There was a decline in penetration efficiency of *E. necator* as leaves aged beyond this leaf position. These results confirmed the expression of leaf ontogenic resistance.

A mechanistic model was constructed, by Bayesian analysis, to quantify the non-linear change in powdery mildew severity as a function of increasing leaf position with different rates of leaf emergence. Two component models separated the effects of leaf resistance and pathogen growth, with the latter component model indicating that the rate and magnitude of pathogen colonisation were significantly different for plants grown in the two different environments. In contrast, there was no significant difference between environments for the parameters of the leaf resistance model, suggesting that the pre-inoculation environment affected the nutritional or some other quality of the plant tissue colonised by *E. necator* rather than having a differential effect on mechanisms of host resistance.

Additional Cabernet sauvignon plants were grown in each pre-conditioning environment and two mature ‘source’ leaves for photosynthates treated with $^{14}C0_2$ for identifying, by autoradiography, the sink or source status of leaves younger than those treated. There was a clear association between the leaf position for maximum severity of powdery mildew and the position of the leaf completing the sink to source transition for shoots exposed to either pre-inoculation environment.

Given that the environment in which primary grapevine shoots develop affects powdery mildew severity, primary shoot development of the most common cultivars grown in Tasmania, Chardonnay and Pinot noir, was quantified over two growing seasons at four commercial vineyard blocks in southern Tasmania. Development of leaves on primary shoots varied widely between and within seasons. Rate of leaf emergence, shoot growth and leaf area development were examined at different positions on the cane, and were highly dependent on ambient temperature, with the plastochron index, shoot length and leaf area increasing linearly with cumulative thermal time. There was a higher rate of shoot growth and leaf area development in the wetter season of 2005-06 than 2006-07 at all sites. For instance at one Chardonnay site, there was an average of 5.62 cm^2 of leaf area emerged per degree day in 2005-06, whereas it was 3.81 cm^2 per degree day the following season. Rate of leaf emergence, calculated from the plastochron index as a function of cumulative thermal time, was the only variable measured which was similar between seasons for each nodal position on the cane, indicating the predictive potential of the linear model. Rate of leaf emergence from shoots arising from different nodal positions on the cane were different, with shoots arising from medial nodes having significantly slower leaf emergence rates than both basal and distal shoots. This effect was consistent, occurring at all but one of the experimental sites.

Results are discussed in terms of practical management of the disease in commercial vineyards in the grape-growing region studied.

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INTRODUCTION

The Australian wine industry contributed over $2 000 million, in 2007-08, to the Australian economy with wine exports being the third largest valued agricultural export commodity behind wheat and beef (ABARE, 2008). Wine regions in Australia can be classified by using the mean January temperature (MJT), the warmest month in the southern hemisphere (Prescott 1965; Smart and Dry 1980). These classifications can be used to guide the location of vineyards, select suitable grape varieties and potential wine styles, and to predict harvest dates (Kirk 1986). Regions in Australia defined by MJT are “cold” (≤ 16.9°C), “cool” (17-18.9°C), “warm” (19-20.9°C) or “hot” (21-22.9°C) (Smart and Dry 1980). Tasmania is considered to be a cold to cool climate for viticulture, which has limited variety selection and methods of pruning and training. With most major commercial development having taken place since the mid 1970’s, the total area planted continues to grow and in 2006 there was a total of 1342 ha, an increase from 459 ha planted in 1997 (Merry and Farquhar 2007). Pinot noir and Chardonnay accounted for 72% of production in 2006, with Sauvignon blanc and Riesling accounting for 17% and the remaining 11% comprising other varieties including Cabernet sauvignon, Merlot, Pinot Gris and Gewürztraminer (Merry and Farquhar 2007). Prior to 2005 almost all wines produced were varietal types suited to still wine production, with sparkling wines tending to be produced from poorly ripened fruit in cool years. Recent years have seen an increase in sparkling wine production from vineyards dedicated to this wine style.

The grape and wine industry in Tasmania contributes less than 1% (Anon 2008) to national production in terms of tonnes of grapes, but has been recognised for the production of premium and ultra-premium wines. This means Tasmania provides a higher proportion to the value of total wine production in Australia, relative to the volume it contributes. Because the cool climate allows production at the premium end of the quality range for Australian wines, vineyard management has tended to concentrate only on quality with yield as a secondary consideration. Indeed, perceived negative effects of higher yields on wine quality (Farquhar 2003) have led growers to manage to low yield targets, with pruning and training methods plus in-season canopy management concentrating on vine balance, and in the red varieties, fruit exposure for colour and flavour development. As a result of these concerns, most commercial vineyards are cane pruned in spite of the reportedly lower production costs for spur pruning and possibilities for mechanisation. Trellising and training systems cover a wide spectrum from simple vertically positioned shoots from two arms of ten bud canes through to various horizontally or vertically split canopy systems.

The Tasmanian wine region is split into several geographically distinct sub regions with the current study carried out in the south east of the island. Although climate is heavily dependent on local factors, overall this area is a little cooler than the northern and eastern subregions and is also markedly drier with an average annual rainfall of 498 mm that ranges from 297 to 735 mm. Rainfall is also highly variable between seasons and all vineyards have irrigation available, usually using micro (dripper) systems. Throughout Tasmania and particularly in this subregion, soils are highly variable (Grose *et al.* 1999) ranging from deep, well drained fertile river terraces to low fertility duplex soils over saline subsoils. This mix of growing conditions results in highly variable vine vigour both within and between vineyards and uncertainties about irrigation and fertilizer management (Wells *et al.* 2007). Because of concerns about fruit quality and excessive vine vigour, growers are reluctant to use nitrogen fertilizers, even those on very low fertility soils, and irrigation is often used only in response to severe stress conditions rather than routine maintenance of optimum water availability. Recent studies (Wells *et al.* 2007) suggest that chronic nitrogen deficiency is widespread in vineyards throughout all the Tasmanian subregions.

Worldwide, grapevine powdery mildew is a prevalent and costly disease, reducing grape yield and affecting wine quality if left uncontrolled (Stummer *et al*. 2005). Tasmania has never had a mildew management strategy developed specifically for local conditions and disease development is promoted by frequent occurrence of high relative humidity and cloudy days, and ambient temperatures that rarely exceed 25^{o}C (Evans 2005). Effective control of powdery mildew can be achieved with multiple applications of a wide array of fungicides (Hall and Wicks 2008), but in Tasmania, poor spray timing and coverage still lead to control failures (Evans 2005). However, there is a need to reduce fungicidal inputs in line with market demands for low or no fungicide residues in wine by using sustainable production methods. Protocols for timing fungicides, according to pathogen activity and/or the susceptibility of various grapevine tissues to powdery mildew, are based on general rules rather than accurate models that reliably predict disease risk. For example, growers have tried to modify recommendations applied elsewhere, for example, the 2, 4, 6 rule applied in the Riverland, which is when fungicides are applied 2, 4, and 6 weeks after budburst, is sometimes extended to 3, 6, 9 (weeks after budburst) in Tasmania to account for slow rates of shoot development (Evans 2005). In addition, high rates of sulfur, commonly 800 to 1,200 g/100 L, are applied to compensate for cool temperatures that can occur at any time (Evans 2005).

In the relatively new wine region of Tasmania, powdery mildew is the most prevalent disease of vines, with serious crop losses often occurring somewhere in the region each growing season. Highly variable disease incidence between vineyards and seasons may be the consequence of variable or inconsistent spray programs as well as variation in the development of both the disease and its host plant with respect to the timing of control actions. The overall objective of the present study was to identify factors associated with grapevine growth and development that influence the risk of powdery mildew. The outcomes are intended to provide a basis for further work to develop effective protection based on an understanding of how the local environment influences the host-pathogen relationship and disease expression.

LITERATURE REVIEW

In this literature review, powdery mildew is considered in terms of its economic importance and management, pathogen lifecycle and disease epidemiology in order to identify knowledge gaps about the interaction between vine and disease development. Host-pathogen interactions and the response of the plant to pathogen invasion, with reference to the powdery mildew fungi, are reviewed for development of hypotheses and discussion about leaf ontogenic resistance to powdery mildew. Further review of the literature is presented in the introduction to each of the research chapters.

Whilst the study clearly includes issues around vine growth and development in response to both environmental conditions and management, it is beyond the scope of the present review to cover the wealth of published information on vine physiology and practical management of vegetative and reproductive growth. Such papers rarely make reference to vine biology in relation to disease incidence, control or management, but specific reference is made to some relevant papers in the experimental chapters of the thesis, particularly Chapter 3. Extensive and detailed discussion of environmental and management influences on growth and development are included in standard texts such as Mullins *et al* (1992) and more recent reviews such as that of Rives (2000) and Vasconcelos *et al.* (2009). Refereed literature used to develop methods are also discussed in the relevant chapters.

POWDERY MILDEW OF GRAPEVINE AND ITS ECONOMIC IMPORTANCE

Like all modern crops, *Vitis vinifera* is cultivated in a monoculture that increases its susceptibility to disease by factors such as genetic homogeneity, uniformity of single clones of the same age and foliage type, and spatial factors, such as dense canopies that reduce airflow and increase relative humidity to create conditions favourable for pathogen proliferation. Diseases of economic importance include downy mildew and botrytis bunch rot, caused by the pathogens *Plasmopara viticola* and *Botrytis cinerea* respectively.

However, the most economically important disease of grapevine worldwide is powdery mildew (Pearson and Goheen 1990), caused by the ascomycete fungus *Erysiphe necator* Schw. ((Braun and Takamatsu 2000; synonym *Uncinula necator*). *E. necator* is a biotroph that infects all green tissues of the vine: leaf blades, petioles, stems, rachises, pedicels, flowers, and immature berries.

Eryisphe necator originated in North America and first appeared in England in 1845 from where it spread rapidly among *V. vinifera* vines cultivated in Europe (Large 1940). Unlike North American species of *Vitis*, *V. vinifera* does not carry any substantial resistance to *E. necator* (Large 1940) and all varieties of *V. vinifera* are comparatively susceptible to powdery mildew, which is now prevalent in all regions of the world where *V. vinifera* vines are cultivated.

Powdery mildew can result in serious losses in grape yield and quality in most years, and total crop loss can occur (Chellemi and Marios 1992; Gadoury *et al*. 2001; Calonnec *et al.* 2004; Savocchia *et al*. 2004; Gadoury *et al.* 2007). Wine made from Chardonnay grapes with as little as 1-5% of bunches affected by powdery mildew can have altered composition and sensory characteristics (Stummer *et al*. 2005). In general, there is a low tolerance of infected grapes in the winery meaning that a high level of disease control is required, usually necessitating multiple applications of protective fungicides.

Crop Protection

The grape and wine industry is highly dependent on the use of fungicides to manage powdery mildew. The most heavily used fungicides in Australia are sulfur and demethylation inhibiting fungicides (DMIs) (Hall and Wicks 2008), however, there is grower and consumer interest in reducing inputs of synthetic pesticides due to economical and environmental pressures (Lichtenberg and Zimmerman 1999). Removal of excess inputs of fungicides is likely to have a range of beneficial effects. For instance, reduction of fungicide usage has been shown to increase the abundance of fungi on the surface of fruit and foliage (Palmer 2009), including some species that are antagonistic toward plant pathogens (Fravel 1988; Cook 1993; Sutton and Peng 1993). Reduced pesticide flows into depleted water catchments, including fungicides formulated with various other chemicals, might also help maintain water quality (Hounslow 1995). Another benefit of pesticide reduction is to reduce long-distance importation of costly synthetic fungicides that consume significant energy during production and are a major source of carbon dioxide emission (West and Marland 2002; Lal 2004). An additional benefit to the grower is that reduction of spray applications throughout the season will decrease costs associated with labour, fuel and fungicide materials.

Sulfur is generally effective for powdery mildew control and has been used for more than 100 years (Large 1940; Bent 1978). Nevertheless, some viticultural sectors, especially some organic growers, are keen to minimise the use of sulfur because of the potential of sulfur to impact negatively on beneficial arthropods (Calvert and Huffaker 1974) and/or the well-being of some vineyard workers (California Department of Pesticide Regulation 2009). Target-specific fungicides can also be prone to development of pathogen resistance, for example the DMIs (Erickson and Wilcox 1997), thus providing another incentive to minimise their use.

Although growers usually commence their spray programs based on careful observations of leaf appearance, subsequent fungicide sprays are often applied at regular intervals, not taking into account temporal variation in the appearance of new, mildew susceptible leaves. To time protective fungicides strategically, a sound knowledge of disease epidemiology, in relation to vine development and environmental conditions, is required. In the interval after a fungicide application, rapid leaf emergence will result in new leaves that are unprotected by fungicide residue. Conversely, application of the subsequent spray might occur when there has been no increase in susceptible leaf area and existing fungicide residues continue to provide an effective cover. In Germany, protective fungicides for downy mildew are timed according to the amount of unprotected leaf area emerged since the last spray (Bleyer *et al*. 2008), based on models that describe shoot development as a function of thermal time (degree days; Schultz 1992). There is potential to use this information to time fungicides for powdery mildew so that the amount of leaf area that is both highly susceptible to infection and at risk of being infected is minimised.

LIFE CYCLE OF *E. NECATOR*

Erysiphe necator overwinters by production of sexual fruiting structures called cleistothecia that produce asci containing ascospores or as resting mycelia in infected buds (Bulit and Lafon 1978; Pearson and Gärtel 1985; Wicks and Magarey 1985). Infected buds give rise to shoots, termed “flag” shoots, which are often completely whitened due to surface colonisation by *E. necator* (Boubals 1961 cited in Pearson and Gärtel 1985). The dominant overwintering fungal structure is related to climate. For example *E. necator* can overwinter as mycelium in buds in Australia, California and Italy but only as cleistothecia in bark crevices or leaf litter in the cold winter climate of New York and Washington states in the USA (Pearson and Goheen 1990; Cortesi *et al*. 2004; Grove 2004; Rumbolz and Gubler 2005; Emmett and Magarey 2008).

Ascospores are released after a minimum of 2.5 mm of rain and when temperatures are 4 – 30°C (Gadoury and Pearson 1990a). Infection is then also temperature dependent and follows release of ascospores with temperatures of 10°C and above (Gadoury and Pearson 1990b). Ascospore infection leads to surface colonisation and epiphytic hyphae and conidiophores that produce one or more conidia in chains. These conidia are dispersed by air to healthy grapevine tissue and the process of conidial infection is described in detail in the next section. Flag shoots produce airborne conidia that disperse the pathogen in the pre-flowering period and they have been observed in Tasmania in October and November (spring in the southern hemisphere). It is notable, however, that the relative importance of primary inoculum from flag shoots or cleistothecia, in Australian vineyards, has never been established. However, flag shoots are thought to be common in most districts of Australia (Magarey 2010).

Conidial Infection

Before infection of the host tissue the spore must adhere to the plant surface. Unlike most other fungi, conidia of the powdery mildew fungi do not require free water for germination and adhesion (Nicholson 1996). Nicholson (1996) suggests that in the barley powdery mildew pathosystem, the conidium exudes a substance to prepare the site for formation of the appressorium prior to infection. It is assumed that appressorial adhesion by *E. necator* occurs in a manner similar to other powdery mildew–host interactions.

The infection process is summarised in Figure 1, as adapted from Hayes (2006). Once the spore has adhered to the host surface (Figure 1 A) a specialised infection structure must develop to penetrate the host epidermal cell for further colonisation of the plant organ (Staples and Macko 1980). Germinating conidia of powdery mildew fungi first produce a primary germ tube from which an appressorium is formed at the tip (Figure 1 B). A penetration peg then develops (Figure 1 B) which does not require an opening (Nicholson 1996) and penetrates directly through the cuticle and cell wall (Ficke *et al*. 2002). The peg then forms a haustorium (Figure 1 C), through which the fungus obtains nutrition from the plant epidermal cell. Secondary hyphae then develop to form secondary infection sites neighbouring the primary infection site (Figure 1 D). Ficke *et al*. (2003) reported that formation of secondary hyphae was evidence of successful establishment of *E. necator* on grape berries. Conidiophore production (Figure 1 D) is observed under ideal conditions around 5–10 days after infection (Delp 1954). Conidia infect juvenile green tissue of susceptible species readily, with infection efficiency declining as the plant organ ages (Doster and Schnathorst 1985; Gadoury *et al*. 2003).

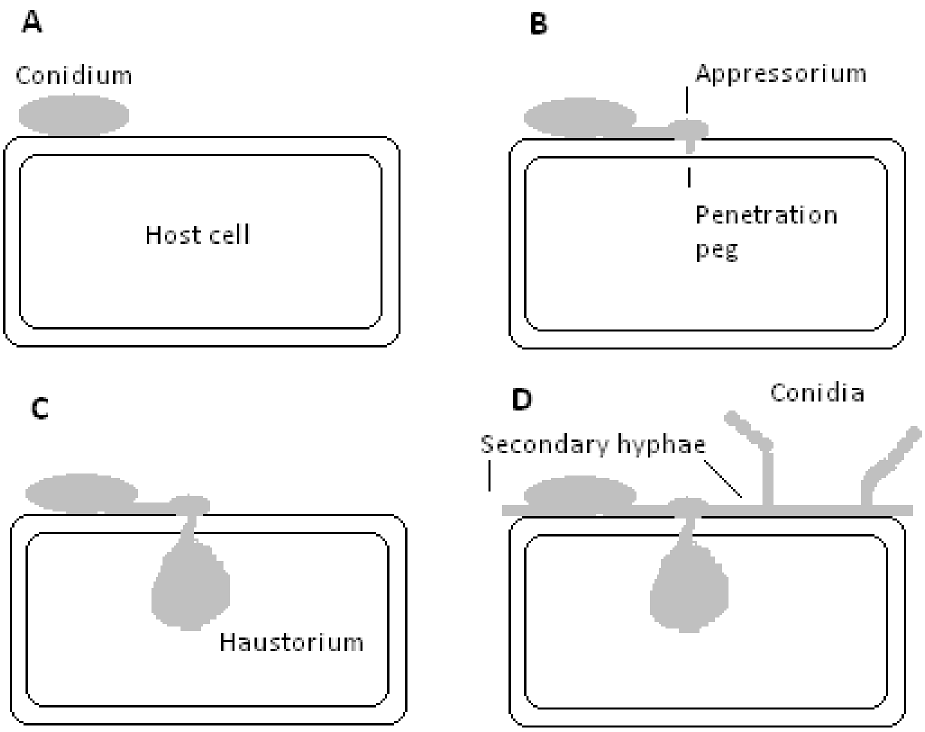

Figure 1. Schematic illustration of the infection process of *E. necator* (modified from Hayes 2006). A conidium at the surface of a host cell (A). The conidium has germinated and has formed an appressorium and penetration peg (B). After the penetration peg has entered the cell, a haustorium, the feeding structure of the fungus, is formed (C). Secondary hyphae, which colonise on the surface of host tissue are then formed along with conidiophores bearing conidia (D). Infections are generally attempted at the junction of anticlinal walls (D.M. Gadoury, Cornell University, pers. comm.).

EPIDEMIOLOGY

Maximum infection of a plant occurs when the environment is favourable, and the host is at a susceptible phenological stage (Agrios 2005). The environment can affect both the susceptibility of the host, for example by inducing stress in a plant, and the activity of the pathogen, for example the temperature for fungal growth. The environment can also be changed by the plant and production system. For example, a dense plant canopy on irrigated vines can provide higher humidity and lower leaf surface temperatures than a more open canopy. The influence of the various factors that affect development of powdery mildew can be modeled by quantifying disease progression in time and space in relation to known environmental influences.

There are two separate but closely related epidemics of powdery mildew occurring on grapevines: one occurs on leaves and the other on bunches. Figure 2 shows the progression of a powdery mildew epidemic in Chardonnay vines grown commercially in southern Tasmania (Evans *et al.* 2005). The frequency and spatial distribution of diseased leaves at flowering has been related to the severity and spatial distribution of powdery mildew on bunches at bunch closure (Calonnec *et al*. 2006). *E. necator* can colonise green floral pedicels and caps (calyptrae) and will infect the developing fruit from these inoculum sources at a high frequency during early fruit set (Gadoury *et al*. 2003). The leaf epidemic influences the amount of inoculum available for infection of highly susceptible flowers and immature fruit (Calonnec *et al*. 2008). The status of the leaf epidemic at flowering depends on when primary infection occurred (Calonnec *et al*. 2008) and the proportion of the leaves in the canopy that were highly susceptible during each infection event. A greater understanding of how leaves vary in their susceptibility to powdery mildew is required in order to quantify variation in the temporal and spatial susceptibility of a grapevine canopy to powdery mildew.

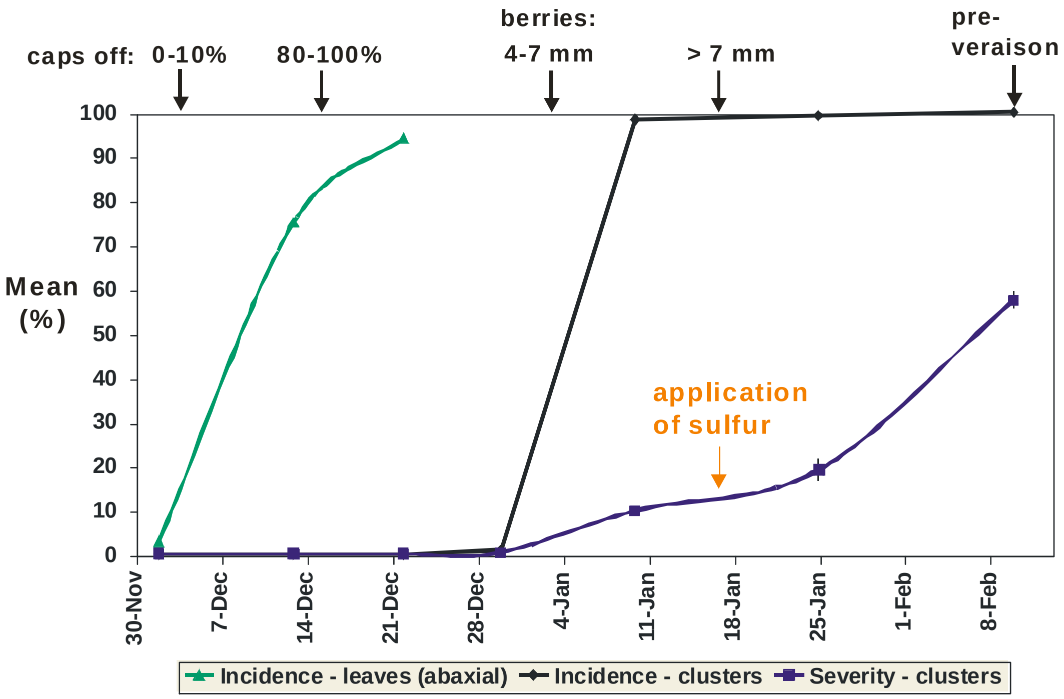

Figure 2. Progression of powdery mildew in small plots of Chardonnay vines that were not treated with fungicide until pea-sized berries. Each point is the mean for six plots. Frogmore Creek vineyard, Coal River Valley, 2004/05 (reproduced from Evans *et al*. 2005).

Knowledge of the location of epidemics in time can aid decision about when to apply crop protection according to pathogen activity. Plant disease epidemics can be predicted from host and environmental conditions, although there is currently no working model for grapevine powdery mildew. A temperature-based decision rule called the Gubler-Thomas (G-T) index (Gubler *et al*. 1999) has been evaluated in Tasmania where it was found that severe epidemics of powdery mildew occurred in growing seasons when the threshold G-T index was never reached (Evans 2005). Accurate models that underpin decisions about timing fungicides strategically are required if strategic spray applications are to be developed.

Disease Resistance

Resistance is the extent to which a plant can withstand, oppose, lessen, or overcome the attack of a pathogen, whereas susceptibility is the extent to which the plant cannot overcome pathogen attack (Kuć 1966). Heath (1996) described six types of disease resistance exhibited by higher plants: parasite-specific, cultivar-specific, nonhost, organ-specific, induced resistance, or age-related. Resistance can be expressed at many stages in the infection process, from inhibition of propagule germination and penetration, to the restriction of colony development after the pathogen has become established.

Leaf age-related resistance

The susceptibility of whole plants or plant organs to disease can change as they age (Agrios 2005). Age-related, or ontogenic, disease resistance has been reported for interactions of perennial hosts with obligate pathogens, such as *Phragmidium violacuem* on blackberries (Evans and Bruzzese 2003), *Phragmidium rubi-idaei* on raspberries (Anthony *et al*. 1985), *Hemileia vastatrix* on coffee (Coutinho *et al*. 1994), *Melampsora larici-populina* on Populus species (Sharma *et al*. 1980) and *Podosphaera clandestina* on cherries (Olmstead *et al*. 2000). Ontogenic resistance can lead to a major reduction in disease severity and may lead to total escape from infection (Ficke *et al*. 2002).

Ontogenic resistance in grapevine leaves is expressed toward two biotrophic pathogens: downy mildew (Reuveni 1998, Kennelly *et al*. 2005) and powdery mildew (Doster and Schnathorst 1985; Singh and Munshi 1993). Doster and Schnathorst (1985) found a gradient in powdery mildew development on grapevine shoots. The older leaves had smaller and fewer colonies compared with younger leaves and there was an inverse relationship between colony growth or sporulation intensity of *E. necator* and leaf maturity. In grape berries, Gadoury *et al*. (2003) observed that resistance to *Erysiphe necator* was acquired within 2–4 weeks after bloom in *V. vinifera*, thus demonstrating that ontogenic resistance in berries occurred much sooner than previously reported by Delp (1954) and Chellemi and Marois (1992). While conidial adhesion, germination and appressoria formation were not affected by berry age, successful formation of the penetration peg and development of secondary hyphae were not observed on older berries (Ficke *et al*. 2003). Whether or not pathogen invasion is halted at the same time during penetration for ontogenically resistant leaves remains to be tested.

Observations of leaf ontogenic resistance in *V. vinifera* and a range of other woody plants suggest aging leaves have one or more mechanisms to inhibit disease development. These may include cell wall and cuticle thickness, nutrient availability in the leaves, and the activity of certain enzymes, such as PR proteins and products of lignin biosynthesis (Reuveni, 1998; Singh and Munshi, 1993). Reuveni (1998) investigated one of these mechanisms further in grapevine and found that leaf ontogenic resistance was associated with the activity of peroxidase and β-1,3-glucanases. Similarly, Giannakis *et al*. (1998) detected an increase in β-1,3-glucanases as well as another PR protein, chitinase, as leaves aged. Accumulation of stilbenes in grape leaves (Schnee *et al.* 2008) and phenolics in grape berries (Ficke *et al*. 2002) have been associated with susceptible tissue, so may also be implicated in ontogenic resistance. However the mechanism/s leading to leaf ontogenic resistance in leaves of woody perennial plants remains unknown. Gee *et al*. (2008) recently screened a range of *Vitis* species for berry ontogenic resistance finding one genotype which failed to develop ontogenic resistance. This offers the possibility of undertaking genetic studies to investigate the inheritance and molecular basis of this powdery mildew resistance character (Dry *et al*. 2010), which may also elucidate the mechanisms involved in berry ontogenic resistance (Gee *et al*. 2008).

PLANT DEFENCE AGAINST PLANT PATHOGENS

In general, plants defend themselves against pathogen attack via two mechanisms: passive defences and active defence responses. Passive defences are constitutively expressed in the host tissue. The active defence responses, which require *de novo* protein synthesis, are regulated through a complex and interconnected network of signalling pathways that mainly involve three molecules: salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). The compounds associated with these mechanisms are summarised in Table 1. For a biotroph such as *E. necator* to infect successfully, it must establish a basic compatibility with its host. Incompatibility between a host and a pathogen results in the recognition of the pathogen and activation of defence mechanisms, while compatibility results in infection (Flor 1971).

Table 1. Putative defence compounds/systems for disease resistance in plants (from Kuć, 2001).

Passive and/or wound responses
Waxes, cutin, phenolic glycosides, phenols, quinones, steroid glycoalkaloids, suberin, terpenoids and proteins (thionins)
Induced after infection
Phytoalexins, reactive oxygen species/free radicals, calcium, silicon/silicates, polyphenoloxidases, peroxidases, phenolics cross-linked cell wall polymers, hydroxyproline and glycine-rich gycoproteins, thionins, antimicrobial proteins and peptides, chitinases, β-1,3-glucanases, ribonucleases, proteases, callose, lignin, lipoxygenases and phospholipases

Passive or constitutive defences

The first line of plant defence to pathogen invasion is passive or constitutive defences such as physical and/or chemical barriers.

Physical barriers

The cuticle, stomata and cell wall are the first barriers to infection for an invading microorganism. In order to penetrate the cuticle, pathogens must either use physical force and/or produce cutin-degrading enzymes (Kolattukudy 1985). The cell wall is a highly organised barrier around the cell and prevents invasion by all microorganisms except those that can overcome this chemical and physical barrier (Vian *et al*. 1996). For pathogens that infect through direct penetration, such as *E. necator*, the thickness of the cuticle can affect the success with which a pathogen invades a host (Martin 1964), though Ficke *et al*. (2004) found cuticle thickness not to be a factor in ontogenic resistance in grape berries.

Chemical barriers

Many plants use secondary metabolites as pre-formed antimicrobial compounds. These “phytoanticipins,” the name suggested by Mansfield (1999), are low-molecular weight antimicrobial compounds that are produced constitutively in anticipation of a pathogen encounter and may allow a plant to slow pathogen invasion while other, slower defensive strategies are induced in response to invasion. Phytoanticipins are similar to phytoalexins, described below, and can be the same compounds but are produced constitutively in the plant, not in response to pathogen invasion.

Pathogen recognition

When the plant’s constitutive defences fail to protect against an invading pathogen an active defence response may ensue. If so, the plant must first recognise that it is being invaded by a foreign body and then respond to these ’elicitors‘. At this stage, plant tissues are able to recognise abiotic or biotic elicitors (Boller 1995) and induce resistance in a process called systemic acquired resistance (SAR) (Ross 1961). SAR can result in induced resistance to bacterial, fungal or viral attack (Kuć 1982). The process is systemic because resistance develops in untreated parts of the plant distal to the initial site of attack. The process of SAR, summarised in Figure 3, results in a broad spectrum resistance.

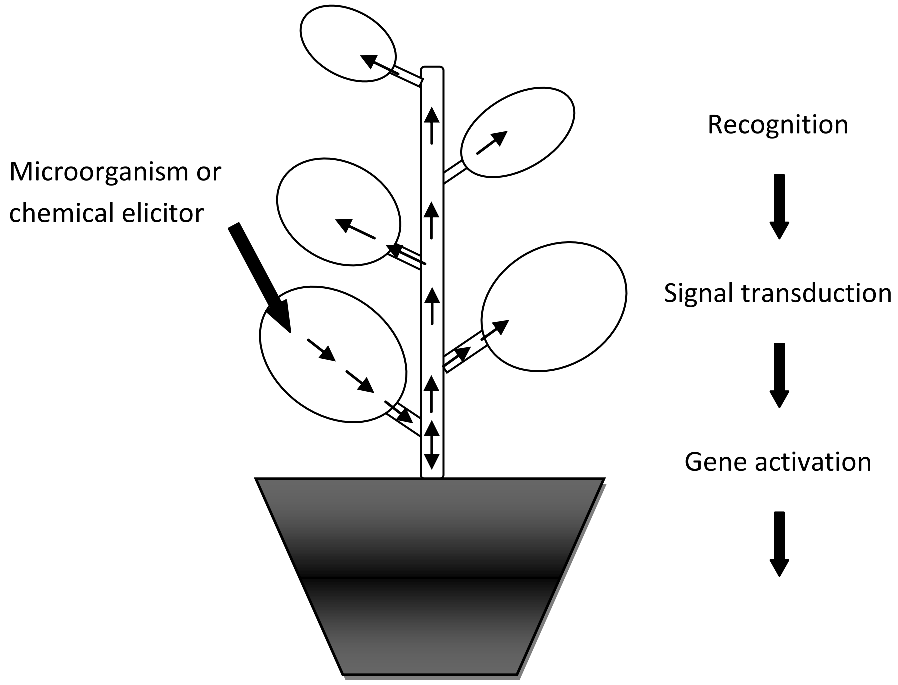

Figure 3. The process of systemic acquired resistance (SAR). The plant first recognises a foreign agent, which causes signal transduction, gene activation and then a series of defence responses. Once these defence responses are activated the plant is then in a "primed state".

A wide range of agents have been reported to induce resistance (Kuć, 2001). Abiotic factors which induce a stress response in exposed tissues, such as heavy metals or UV light, can also induce resistance to infection (Chappell and Hahlbrok 1984). For example, Yildirim *et al*. (2002) found that KH_2PO_4 applied to leaves induced resistance in grapevine bunches against powdery mildew. Biotic elicitors include cell wall fragments released from fungi and bacteria, hydrolytic enzymes of plant or pathogen origin, some peptides, glycoproteins and polyunsaturated fatty acids. For instance chitosan, a chitin derivative formed from components of arthropod exoskeletons by deacetylation has been shown to induce a defence response in grapevine against *B. cinerea* (Barka *et al*. 2004; Compant *et al*. 2005).

Often, elicitors are non-specific and simply alert the plant cell that it has been damaged in some way. However, there are also specific elicitors that enable pathogen-specific defence, which is conditioned by avirulence and resistance genes in a gene-for-gene relationship (Flor 1971). The interaction of gene transcripts of the pathogen and host initiates a signal transduction cascade that activates an array of defence responses (Hammond-Kosack and Jones 1996). This type of resistance is expressed in a wild north American grapevine species, *Muscadinia rotundifolia*, which has been shown to be resistant to a number of economically important diseases of grapevine, including *E. necator* (Olmo 1986). The resistance to *E. necator* in *M. rotundifolia* has been found to be controlled by a single, dominant gene termed *Run*1 (Resistance to *Uncinula necator* 1; Bouquet 1986; Pauquet *et al*. 2001). Currently, a map-based cloning approach is being used to isolate *Run*1 (Barker *et al*. 2005) and to examine the potential for introducing it to *V. vinifera* cultivars to try and introduce significant and durable resistance (Dry *et al*. 2010).

Rapid active responses

There are an array of cellular defence responses and metabolic changes in a plant cell in response to recognition of the pathogen. These mechanisms may or may not act in a coordinated manner. The success of active resistance depends on both the rapidity and the intensity of the plant response to infection.

Cell wall modifications

The cell wall is the first barrier to pathogen penetration and reinforces plant defence against wall-degrading enzymes produced by pathogens (Hammerschmidt and Nicholson 1999). The cell wall acts as a mechanical barrier to physical penetration toward the protoplast (Baysal *et al*. 2003), a diffusion barrier against toxins (Hammerschmidt and Nicholson 1999) and can be a major obstacle to the activities of lytic fungal enzymes (Busam *et al*. 1997). If the host cell can repair and reinforce its cell walls quickly enough, it might reduce the penetration efficiency of the pathogen.

Several types of reinforcement are produced by host cells. The first step in cell wall modification is the increased acetylation of cell wall polysaccharides, which is catalysed instantly (Matern and Grimmig 1994). Peroxidases are the main enzyme involved in cell wall modifications; they catalyze the suberization and lignification of cell walls (Dixon and Harrison 1990). Lignification and suberisation of tissues progresses gradually and this type of defence takes several days (Matern and Grimmig 1994). The deposits of lignin may be highly localised and block the movement of hyphae into the cell or the whole cell may become lignified and trap the pathogen (Hammerschmidt and Nicholson 1999).

Cell wall reinforcement tends to occur to a greater extent and form more rapidly in resistant hosts than in susceptible hosts. Moreover, inhibition of the production of callose or lignin synthesis by the pathogen enhances its penetration efficiency. Deposits between the plant plasma membrane and cell wall are referred to as papillae. Papillae are deposits directly below the point of attempted penetration and are made of callose, silicon, lignin and proteins (Aist 1976). In a subsequent paper, Aist and Israel (1977) suggested that the effectiveness of papillae, a physical and/or chemical barrier to fungal invasion, depends on the time of formation. Ficke *et al.* (2004) found papillae to be formed in grape berries after unsuccessful attempts at infection, and to a greater degree in susceptible tissue. Whether papillae are involved in ontogenic resistance in grape leaves remains to be investigated.

At the membrane

The host membrane appears to be involved in the earliest stages of pathogen recognition and signal transduction (Mathieu *et al*. 1991). A change in membrane permeability after exposure to a pathogen causes fluxes in ions, such as K^+, H^+ and Ca^{2+} (Mathieu *et al*. 1991; Thuleau *et al*. 1994), and results in changes to gene activation and the triggering of the defence responses. Pathogens can also increase the production of reactive oxygen species such as hydrogen peroxide (H_2O_2), the hydroxyl radical (-OH) and the superoxide anion (O_2^-) termed the "oxidative burst" (Hammerschmidt and Nicholson, 1999). This oxidative burst can coordinate an assorted set of defence responses in plants (Aziz *et al*., 2004): it triggers signals that affect gene expression (Lamb and Dixon 1997), strengthen plant cell walls through crosslinking reactions (Thordal-Christensen *et al*. 1997) and it also initiates later defence responses (Hammerschmidt and Nicholson, 1999). The reactive oxygen species at the site of infection are also produced in quantities capable of killing micro-organisms directly (Apostol *et al.* 1989; Legendre *et al.* 1993; Walters 2003; Custers *et al.* 2004).

The hypersensitive response

The hypersensitive response is a programmed, localised and rapid cell death of one or a few host plant cells in response to invasion by a pathogen (Hammerschmidt and Nicholson 1999). The response can be very effective against obligate parasites, as they require living host cells for nutrition (Hammerschmidt and Nicholson, 1999). This programmed cell death is triggered by cell membrane responses discussed above.

Phytoalexins

Phytoalexins are low molecular weight secondary metabolites synthesised in response to infection and physiological stress by most flowering plants (Hammerschmidt 1999). When produced in high enough concentrations, many have the capacity to inhibit or slow the advance of fungal hyphae (Creasy, 1999). However as Kuć (1994) indicated, the extent to which phytoalexins determine resistance is obscured by many other contributing factors.

Phytoalexins are a diverse group of compounds, which differ from one plant family to another (Hammerschmidt and Nicholson, 1999). The presence of these compounds may have a significant effect on the success or failure of an infection attempt (Creasy 1999). Hammerschmidt and Nicholson (1999) proposed that incompatible reactions between host and pathogen give rise to the rapid *de novo* synthesis and accumulation of these compounds. These authors reported a specific response of cells of resistant sorghum plants to incompatible fungi which involved production of small inclusion bodies near the site of synthesis of the phytoalexins. These inclusion bodies then migrated into the infecting hyphae where phytoalexin concentrations in these cells surpassed what was required to kill the pathogen *in vitro*. These events did not occur in susceptible host-pathogen interactions.

Stilbenes are phytoalexins considered fungitoxic and are synthesised both in the leaves and berries and can be induced by methyl jasmonate (Larronde *et al*. 2003) and other biotic and abiotic stimuli (Pezet *et al*. 1991). The capacity, intensity and rapidity of grapevines to produce stilbenes has been proposed to be indicators of the plant's resistance to fungal infection (Pezet *et al*. 1991) and they have been shown to be fungitoxic against grape pathogens causing botrytis bunch rot (*Botrytis cinerea*) and downy mildew (*Plasmopara viticola*) (Langcake and Pryce 1976). In grapevines, the most studied stilbene-type compound is resveratrol. Viniferins, which are dimers of resveratrol (Pezet *et al*. 2004), have been shown to be a reliable marker for resistance to powdery mildew and for assessing the defence potential of grapevine cultivars (Schnee *et al*. 2008).

Delayed active defences

Delayed active defenses include further containment of the pathogen, wound repair, expression of pathogenesis-related proteins and systemic acquired resistance. These mechanisms restrict the spread of the pathogen after infection is established and contain the damage to cells infected.

Pathogenesis-Related (PR) proteins

Pathogenesis-related (PR) proteins are a group of proteins that are induced and accumulate locally and often systemically in the plant in response to infection (Hammerschmidt and

Nicholson 1999). PR proteins were first described in the leaves of tobacco plants following infection with tobacco mosaic virus (Van Loon and Van Kammen 1970). PR proteins might also play a role in growth and development as they are sometimes present at low levels constitutively (Giannakis *et al*. 1998). For instance the thaumitin-like PR proteins and chitinases are present in increasing levels in healthy grape berries from veraison onwards (Pocock *et al.* 2000).

Accumulation of PR proteins has been reported in grapevine leaves and berries in response to infection by the causal agents of powdery and downy mildew (Bézier *et al*. 2002; Ficke *et al*. 2004), although no specific relationships with ontogenic resistance have been reported. Grapevine tissues having constitutively expressed PR genes might be more resistant to pathogen attack (Jacobs *et al*. 1999). Giannankis *et al*. (1998) found that grapevine genotypes with high constitutive levels of these enzymes always had a low resistance rating to powdery mildew. Also, there was an increase in the level of these enzymes after wounding or infection with powdery mildew. The PR proteins found in grapevine, β-1,3-glucanases and chitinases, have been reported to have direct antimicrobial activity *in vitro* by degrading cell wall components (Bézier *et al*. 2002; Baysal *et al*. 2003; Hofgaard *et al*. 2005).

Co-ordination of defence responses

Plant defence is attributed to both the constitutive mechanisms and active mechanisms induced by the invading pathogen. The success of active resistance depends on both the rapidity and the intensity of plant response to infection. The specific interaction between host and pathogen is crucial to the success of the resistance or the pathogen invasion, and is mediated by many pathways involved in producing or detecting elicitors, enhancers, suppressors and secondary signals.

APPROACH AND STUDY OBJECTIVES

Erysiphe necator is the casual organism of grapevine powdery mildew, a serious disease which left uncontrolled can affect grape production and wine quality. The asexual (conidial) stage of *E. necator* does not require free water for infection, growth and reproduction, which means that powdery mildew can occur in Tasmanian vineyards every year, even when precipitation is very low. The recommended method of controlling grapevine powdery mildew is multiple, calendar-based applications of protective fungicides throughout the growing season; however, this does not take into account variation in the expression of ontogenic resistance that is inherent in a proportion of grape leaves. Interactions between woody plant species and biotrophic fungi were reviewed in order to describe how whole plants or plant organs of varieties of *V. vinifera* and other *Vitis* species resist these pathogens. From the review there appears to be scope to manipulate the timing of spray applications by taking into account the heterogeneity of foliage and fruit susceptibility to disease development. Consequently, the overall objective of this project was to quantify leaf ontogenic resistance in relation to shoot growth and leaf development and environmental factors that influence leaf development and hence ontogenic resistance. Such knowledge may aid assessment of the proportion of all leaves on a grapevine shoot that are susceptible to infection by *E. necator* and how that proportion varies during the growing season (Calonnec *et al.* 2008). Knowing what environmental or vine factors influence the relative severity of powdery mildew on a leaf and the capacity of that leaf to respond to novel control methods, such as elicitors of induced disease resistance, will allow for more targeted management of powdery mildew.

Field experiments were carried out using Pinot noir and Chardonnay because these are the main varieties grown in Tasmania. A different variety, Cabernet sauvignon, was selected for controlled-environment experiments in the glasshouse because of the availability of clean, dormant canes for propagation of healthy plants and for reliable dual culture of the plant and pathogen (Evans *et al.* 1996).

The following research chapters have been structured into discrete units in preparation for publication. Each chapter is linked to the previous chapter through the opening paragraph and the entire body of work is interpreted in the General Discussion. Given that the results of the glasshouse and field experiments cannot be related directly because different grapevine varieties were used, the aim of the General Discussion was to further interpret the individual studies for their significance in describing the host-pathogen interaction.

CHAPTER 1 – ONTOGENIC RESISTANCE IN GRAPEVINE LEAVES

INTRODUCTION

Erysiphe necator is a biotrophic fungus that causes powdery mildew when it colonises green tissues of members of *Vitaceae* (Bulit and Lafron 1978), including the widely cultivated grapevine, *Vitis vinifera.* The fungus penetrates the host epidermis directly and forms a haustorium that derives nutrients from a functioning plant cell to support growth of epiphytic hyphae and reproductive structures (Struck and Mendgen 1998). According to Hawksworth *et al*. (1995), resistance is "the power of an organism to overcome, completely or in some degree, the effect of the pathogen or other damaging factor". Six types of disease resistance exhibited by higher plants were defined by Heath (1996): parasite-specific, cultivar-specific, nonhost, organ-specific, induced, or age-related resistance. This last type of resistance, also called ontogenic resistance, refers to the condition whereby whole plants or plant organs become more resistant to pathogen colonisation as they age, relative to the plant or organ age when colonisation is at its maximum level (Heath 1996).

Among biotrophic pathogens that infect green tissue of woody, perennial plants, changes in the extent of pathogen colonisation according to leaf position are common. Lesion or pustule number is a common macroscopic sign of disease affected by leaf position (e.g. Schwabe 1979; Sharma *et al*. 1980). The decline in lesion numbers as leaves become older is presumably an expression of ontogenic resistance, although events occurring at the microscopic or biochemical level have been reported infrequently. In a series of experiments conducted at constant temperature, Doster and Schnatorhorst (1985) inoculated leaves at different positions on shoots of various cultivars of *V. vinifera*. The percentage of germinated *E. necator* conidia that developed secondary hyphae 48 h post inoculation declined as grapevine leaves matured beyond a midvein length of 3-5 cm. There was also a corresponding decline in colony hyphal length. By definition, the older leaves were inhibiting fungal development and were expressing ontogenic resistance, although the cause of this phenomenon was not investigated. Differences in fungal development according to leaf position on a plant shoot have also been observed for the biotrophic rust fungi *Uromyces phaseoli* infecting French beans (*Phaseolus vulgaris*, Heath 1981) and *Hemileia vastatrix* infecting *Coffea* spp. (Coutinho *et al*. 1994).

Very little is known about the mechanism/s of leaf ontogenic resistance, which might be expressed pre- or post fungal penetration of host tissue. If ontogenic resistance is expressed post penetration, then further colonisation of the plant tissue might be inhibited by induction of a defence response (Kuć 1982) and/or the tissue becomes less susceptible to subsequent infection by the same pathogen (Ficke *et al*. 2002). In the downy mildew-grapevine pathosystem, leaf ontogenic resistance was correlated positively with increases in peroxidase and β-1, 3-glucanase activities (Reuveni 1998). These enzymes may be made in advance of, or in response to, pathogen invasion. Enhanced peroxidase activity is a marker for leaf senescence (Thomas and Stoddart 1980; Takahama *et al*. 1999) and enzymes associated with leaf senescence may also be involved in plant defence mechanisms against pathogens (Lamb and Dixon 1997). Therefore, it would be difficult to separate biochemical effects that relate both to leaf senescence and pathogen defence. Another approach is to understand ontogenic resistance in grapevine leaves as the proportion of all leaves on a grapevine that are highly susceptible to infection by *E. necator* varies during the growing season (Calonnec *et al*. 2008). Understanding and predicting which leaves develop the most powdery mildew could aid decisions about disease management.

The rate of growth in all biological systems is influenced by temperature. For example, temperature determines the rate of leaf emergence (Schultz 1992) and is a variable that can be used in controlled experiments to manipulate shoot growth. The first step in characterising leaves susceptible to powdery mildew is to quantify the relationship between powdery mildew severity and leaf position precisely, under conditions that are optimum and sub-optimum for growth of *V. vinifera* prior to inoculation with *E. necator* and incubation conditions that promote optimum fungal development. The optimum temperature for *E. necator* growth and reproduction is around 25°C (Delp 1954; Chellemi and Marois 1991), while the optimum temperature for grapevine growth is 25°C (Winkler 1970; Jackson 2008). Temperature not only affects the growth of the pathogen and the host but also how the plant reacts to the pathogen. When plants are exposed to high or low temperature before inoculation, their susceptibility to several pathogens may be increased or decreased (Yarwood 1959). For instance, treatment of plants with short heat pulses (Stermer and Hammerschmidt 1987; Abbattista Gentile *et al*. 1988; Vallelian-Bindschedler *et al*. 1998) or cold hardening (Gaudet and Chen 1987; Ergon and Tronsmo 2006; Moyer *et al*. 2009) can lead to induced resistance against a range of pathogenic fungi. The majority of temperature stress studies are performed under controlled conditions using constant temperature and these conditions do not reflect the variable conditions that occur in the field. Nevertheless, these studies show how environmental conditions can act directly on plant tissue to alter its susceptibility to infection by fungal pathogens.

The relationship between leaf position and disease severity or any other component of disease can be determined by fitting different linear or non-linear models (Ratkowsky, 1989) to obtain a model of statistical 'good fit' or one that has parameters that can be interpreted biologically. A Bayesian approach to statistical inference (Berger 1993) was investigated for its potential to describe how environmental conditions, such as temperature prior to infection, influenced the spatial distribution of powdery mildew severity across leaves of a grapevine shoot. Bayesian analysis is performed by combining the prior information ($\pi(\vartheta)$) and the sample information (x) into what is termed the posterior distribution of ϑ given x (or 'posterior' for short), from which all decisions and inferences are made (Berger 1993). Until recently, the Bayesian approach to statistical inference was restricted to theoretical problems with limited application by specialists in the field of biometrics. Since the 1990s, applications have expanded due to an increase in computational capacity that has made more practical the complex mathematical calculations required. In addition, the availability of Markov Chain Monte Carlo (MCMC) methods has greatly simplified the computation of 'posteriors' compared to the corresponding classical tools (Brooks 1998). Consequently, the use of Bayesian statistics in plant pathology has become more common in recent years (Mila and Michailides 2006). The Bayesian approach includes prior information within the model in addition to biological data derived empirically. This approach allows models to be more easily selected, since there is less need to have models which require the calculation of frequencies to determine probabilities (Rubin 1984). Additionally, the Bayesian approach allows the calculation of the probability of any number of hypotheses, based on the posterior distribution, whereas the classical approach of multiple hypothesis testing is more complex (Berger 1993).

The first aim of this research was to quantify the relationship between the severity of powdery mildew and the position of leaves on shoots of *V. vinifera* variety Cabernet sauvignon that had developed, prior to inoculation with *E. necator*, in two different environments. Temperature was used to create environments that were either near optimum or sub-optimum for the rate of leaf emergence. The aim was to identify the leaf position on shoots of Cabernet sauvignon vines expressing maximum severity of powdery mildew and to evaluate the Bayesian approach to statistical inference for interpreting the influence of the two different environments on grapevine shoot development and subsequent expression of powdery mildew. The second aim was to quantify the relationship between leaf position of this same grapevine variety and the percentage of conidia germinated and the percentage of conidia with secondary hyphae when shoots had developed at optimum temperatures prior to inoculation with *E. necator*. The aim was to identify any changes in fungal development associated with infection that varied according to leaf position.

MATERIALS AND METHODS

Plant material and conditions prior to inoculation

Own rooted grapevines (*Vitis vinifera* L.) cv Cabernet sauvignon, clone Q390-05, were grown in 15 cm-diameter pots in a glasshouse with a 16 h day length supplemented by 400 W mercury halide lights. Dormant vine cuttings were obtained from South Australian Vine Improvement Inc. (SAVII), rooted in moistened vermiculite above a heated bed and pruned to one bud at the time of planting and grown until there were approximately 20 nodes. Developing inflorescences were removed as soon as they became visible. Two separate experiments were carried out to obtain vines with different rates of leaf emergence. Ten plants were grown at 25°C (± 5°C) and then the experiment was repeated in the same glasshouse except that eight plants were grown at 18°C (± 8°C). These two preconditioning environments were intended to produce relatively fast (near optimum) or slow (sub-optimum) rates of leaf emergence at the time of inoculation. Throughout the pre-treatment period, shoots were maintained free from powdery mildew using vapours of penconazole (Topas®, Syngenta Crop Protection Pty Ltd) as described by Szkolnik (1983).

During pre-treatment, growth rate was measured as rate of leaf appearance for each temperature regime. Lamina lengths on all leaves of each shoot were measured every 3-4 days until treatment for the calculation of the plastochron index (PI, Erickson and Michelini 1957, and Chapter 2 for more detail). A reference length of 30 mm was chosen based on earlier results in grapevine (Freeman and Kliewer, 1984; Schultz 1992). The PI denotes the number of leaves on a shoot. The rate of leaf emergence for each shoot was calculated from linear regressions of PI against calendar day or cumulative thermal time as degree days above a minimum temperature of 10^{o}C (Winkler 1970). A *t* test was used to test whether or not the means for leaf lamina length of fully expanded leaves, number of leaves emerged per day or emerged per unit of thermal time for each pre-treatment environment were significantly different.

Inoculation method for assessment of disease severity

Topas® was removed from the glasshouse 7 days prior to inoculation with *E. necator*. Immediately before inoculation the glasshouse was re-set to 25°C for the plants pre-treated at 18°C. Leaves were numbered at the time of inoculation according to position from the apex, starting from leaf position 1 for the first leaf with a lamina length ≥ 30 mm, and then 2, 3, etc, for older leaves. Leaves at leaf position 0-2, 3-5 or ≥ 6 were classed as immature, expanding or mature, respectively, indicating that leaves in each category had expanded to approximately <50%, 50 to 90% and >90%, of their mature size.

Erysiphe necator was collected, as a bulk isolate, from a vineyard in southern Tasmania (42°48'33"S, 147°25'37"E) and cultured on detached leaves of cv. Cabernet sauvignon as described by Evans *et al*. (1996). Conidia were shaken off leaves of 12-day old cultures into distilled water containing 0.05% Tween 20. The conidial suspension was adjusted to approximately 10^5 conidia per ml water with the aid of a hemacytometer and applied to the adaxial side of all leaves using a hand held atomizer (Preval® sprayer power unit, Precision Valve Corporation), according to the method of Gadoury *et al*. (2001). Leaves were dried of visible moisture with a domestic fan immediately after inoculation. After 14 days, disease severity per leaf, defined as percentage of leaf area colonised by *E. necator*, was determined with the aid of a standard area diagram (B. Emmett, Department of Primary Industries, Victoria, pers. comm.).

Construction of Bayesian model

Independent mathematical models of two biological processes were constructed to describe powdery mildew severity on individual grapevine leaves at different positions on the shoot. The processes modelled were pathogen growth and leaf resistance to powdery mildew. Pathogen growth was modelled as a hypothetical situation where the plant organ does not resist pathogen infection. A logistic growth response was assumed whereby the area of leaf colonised by *E. necator* increased exponentially with time, before reaching a maximum rate of colonisation after which the rate slowed in proportion to the declining leaf area and nutrients available for colonisation. The leaf resistance model described the reaction of a leaf to suppress pathogen colonisation, according to leaf position. Again, it was assumed that the rate of suppression of pathogen colonisation increased to a maximum level with increasing leaf position, after which this rate declined until macroscopic signs of the pathogen were no longer visible. This model was based on the inverse-logistic model developed by Gomez (2005) for the blackberry rust pathogen, *Phragmidium violaceum*. These concepts were provided to R. Corkrey, University of Tasmania, who expressed them mathematically and conducted the Bayesian analysis in consultation with A. Merry. The equations and Bayesian analysis are described below.

Pathogen growth model

The proportion of leaf area covered by powdery mildew was estimated by the logistic model:

$$a_{jp} = 1/(1 + \exp(\beta_j - \gamma_j p)) \qquad (1)$$

where a_{jp} was the proportional area colonised by mildew on leaf position (p) on plant (j), and γ_j and β_j were constants to be estimated. The leaf position was the ordinal series p = 1,2,…p_j in which p = 1 had a lamina length ≥ 30 mm and the length of the series could differ between plants. The condition $\gamma_j > 0$ was imposed to ensure that a_{jp} increased with leaf position. The constant γ_j was an indicator of the rate of colonisation and β_j indicated the magnitude of colonisation.

Leaf resistance model

Leaf resistance to powdery mildew was estimated as:

$$s_{jp} = 1 - (1 - \exp(-p\delta_j))^{\varepsilon_j} \qquad (2)$$

where s_{jp} was the disease resistance for leaf position p on plant j, and δ_j and ε_j were constants to be estimated. In this model, the magnitude of s_{jp} declined as the leaf position, p as defined for equation (1), increased, meaning that resistance increased as leaves aged. The conditions $\delta_j > 0$ and $\varepsilon_j > 0$ were imposed to ensure that the s_{jp} declined with leaf position and that s_{jp} was positive. The constant δ_j indicated the rapidity of the disease resistance response with increasing leaf position and ε_j indicated the position at which leaves expressed an equivalent level of disease resistance.

Overall model

Growth of *E. necator*, as measured by disease severity, was described stochastically using the Normal distribution in which the mean was given by the product of the two models:

$$m_{jp} \sim \mathrm{N}(a_{jp} \times s_{jp}, \tau_m) \qquad (3)$$

where τ_m was the reciprocal variance or precision.

In Bayesian analysis, parameters are treated as if they were random variables with probability distributions (Gelman *et al*. 1996). There are three key components associated with parameter estimation: prior distribution, likelihood function, and posterior distribution. The adjectives ‘prior’ and ‘posterior’ are relative terms relating to the observed data (Winkler 1972). ‘Priors’ were specific to the parameters β_j, γ_j, ε_j, δ_j, and τ_m. ‘Prior’ parameters use existing evidence about the parameters of interest and can be summarized quantitatively via probability distributions called the ‘prior’ distribution

(Gelman *et al*. 1996). The parameters were grouped according to the component of the model in which they appeared. The 'priors' used and their associated parameters are summarised in Table 1.1.

Table 1.1 'Priors' used in model construction and respective distributions. Hierarchical 'priors' were used to allow for variation between plants. N refers to the normal distribution and Ga represents the Gamma distribution (Gelman *et al*. 1996).

Model	Prior	Distribution
Pathogen growth	β_j	N (β^*_i, τ^β)
	γ_j	N (exp(γ^*_i), τ^γ)
	β^*_i and γ^*_i	N(0, 0.01)
	τ^β_i and τ^γ_i	Ga(0.01, 0.01)
Leaf resistance	δ_j	N(exp(δ^*_i), τ^δ)
	ε_j	N(exp(ε^*_i), τ^ε)
	δ^*_i and ε^*_i	N(0, 0.01)
	τ^δ_i and τ^ε_i	Ga(0.01, 0.01)
Overall	τ_m	Ga(0.01, 0.01)

After assigning 'priors', the full joint distribution could be determined as the product of the likelihood and the associated 'prior' distributions. The resulting 'posterior' was complex and of many dimensions and so inference was obtained in the form of posterior means and variances obtained via Markov Chain Monte Carlo (MCMC) simulation (Brooks 1998). Implementation was chosen in which each parameter was updated in turn using either an Adaptive-within-Gibbs algorithm as described by Roberts and Rosenthal (2001) or Gibbs update (Brooks 1998), depending upon the form of the associated posterior conditional distributions. All simulation software was written in FORTRAN 95 by R. Corkrey, University of Tasmania. The model was run for 100,000 iterations. The sample may not be representative of the probability distribution, and, to address this problem, a number of the parameters resulting from the first iterations were discarded in a process called a 'burn-in' (Jensen 2001). A 50% burn-in was used to ensure the model distributions converged, to reach the desired target distribution of *x*. Sensitivity studies and standard diagnostic techniques were used (Brooks and Roberts, 1998) to assess model validity.

Inoculation method for quantifying early pathogen colonisation

The relationship between leaf position and the percentage of germinated conidia and the percentage of conidia with secondary hyphae was examined using light microscopy. Ten plants were grown at the 25°C environment from own-rooted vines as described for the experiment where the macroscopic colonisation by *E. necator* was assessed. Conidia of *E. necator* were multiplied as described above and transferred to the adaxial side of each leaf along the shoot using an artists' paint brush. Plants were then maintained in the glasshouse at 25°C as described previously. After 72 h, a 2 x 4 cm section of leaf was cut adjacent to the basal end of the midvein of each leaf. Sections were cleared in 3:1 ethanol: glacial acetic acid for 48 h, softened in lactoglycerol for 24 h and stained with lactoglycerol with 0.1% trypan blue for 48 h, according to a method modified from Carver and Ingerson-Morris (1989). Sections were examined at 400X magnification and the first 40 conidia observed per section were designated into three classes: ungerminated, germinated with a primary germ tube but no secondary hyphae, or germinated with secondary hyphae. The presence of one or more secondary hyphae per germinated conidium was used to indicate that infection had proceeded to penetration of the leaf cuticle and formation of haustoria (Ficke *et al*. 2003). For each plant and leaf position, the overall germination, the percentage of germinated conidia with a primary germ tube (only) or with secondary hyphae was calculated and plotted against leaf position. A natural spline curve was fitted to the data (Green and Silverman 1994).

Statistical estimation of leaf position expressing maximum pathogen infection and maximum severity of powdery mildew

Nadaraya-Watson kernel regression (Nadaraya 1964; Watson 1964) was used to smooth the relationship between leaf position of each shoot and powdery mildew severity or percentage of conidia with secondary hyphae. A bootstrap approach (Efron and Tibshirani 1998) was used to identify the leaf positions at which disease severity or percentage of conidia with secondary hyphae was highest; that is, the leaf position mode. Each data set was bootstrapped 1000 times using the sample function in the R software package and then the mode calculated by the hrm function (Poncet 2009) also in the R software package (R Development Core Team 2009). A *t* test was used to identify any difference between the mean mode for the leaf position for maximum disease severity and leaf position for maximum pathogen infection, as estimated by conidia with secondary hyphae.

RESULTS

Leaf appearance before inoculation

There was a significant ($P < 0.05$) difference in the rate of leaf emergence on shoots of plants exposed to different environments prior to inoculation with *E. necator* (Table 1.2). On average, 0.54 leaves emerged per day at near-optimum temperatures for shoot growth, while only 0.23 leaves emerged per day at sub-optimum temperatures. Hereafter, the two pre-inoculation environments are referred to as 'near optimum' or 'sub-optimum' for the rate of leaf emergence. When the rate of leaf emergence was calculated per unit of thermal time, there was no significant difference ($P > 0.05$) between the two groups of plants: the mean rates were 0.034 and 0.035 leaves emerged per degree (°C) day for the near optimum and sub-optimum pre-treatment conditions, respectively (Table 1.2). At the time plants were inoculated and transferred to the 25^{o}C glasshouse, there was no significant difference ($P>0.05$) in leaf lamina length of leaves that had fully expanded between the two pre-treatment environments. Mean lamina lengths were 91 mm (sd =11) and 88 mm (sd = 8), for the 18°C and 25°C glasshouses respectively.

Table 1.2. The mean number of leaves emerged per day and mean number of leaves emerged per unit of thermal time (degree (°C) day) for shoots of individual plants grown in different environments prior to inoculation.

	Mean number of leaves emerged	
Average pre-inoculation temperature (°C)	Per day	Per degree (°C) day
18	0.23	0.034
25	0.54	0.035
P value	<0.001	ns

Disease expression according to leaf position

No powdery mildew was observed at leaf positions ≥ 17 on shoots with the higher rate of leaf emergence per day (Figure 1.1A), nor at leaf positions ≥ 11 for shoots with the lower rate of leaf emergence per day (Figure 1.1B). In general, the mean severity of powdery mildew on leaves for a given leaf position where powdery mildew was observed was higher at the optimum growth rate (Figure 1.2A) than at the sub-optimum growth rate (Figure 1.2B). The modal leaf position at maximum disease expression occurred on average at leaf positions 4.4 and 3.7 for near optimum and sub-optimum pre-inoculation environments, respectively (Table 1.3). For both pre-inoculation environments, disease expression was more severe on expanding leaves (positions 3-5) than on immature (positions 0-2) or mature leaves (position 6 or higher; Figure 1.2 & Figure 1.3). Disease severity decreased as leaf maturity increased beyond leaf position 3-4 (Figure 1.2 & Figure 1.3).

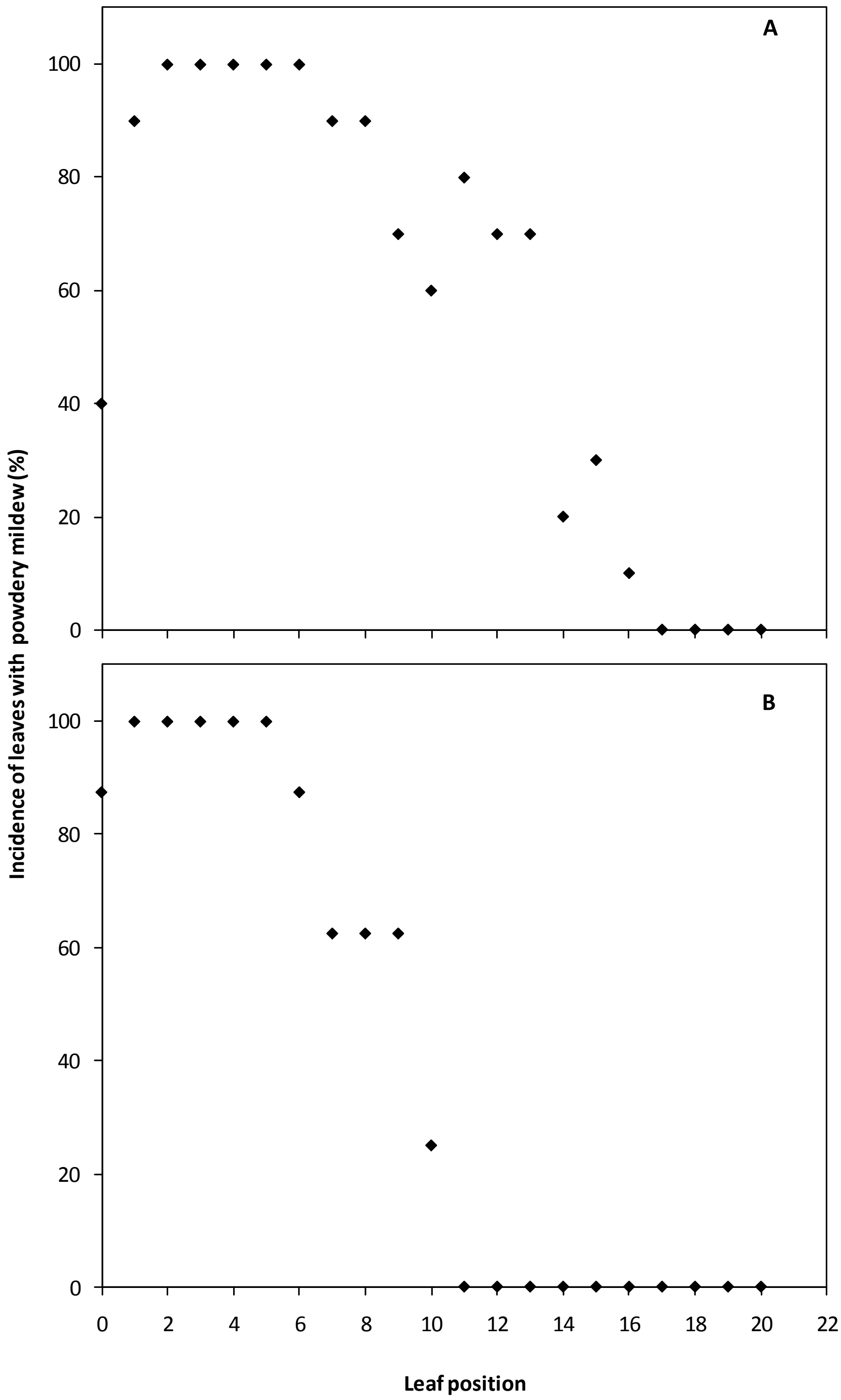

Figure 1.1. The effect of leaf position on shoots of glasshouse-grown Cabernet sauvignon vines on incidence of powdery mildew 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Leaf position increases with increasing leaf maturity. Plants grown at an average of 25°C (A) or 18°C (B) prior to inoculation.

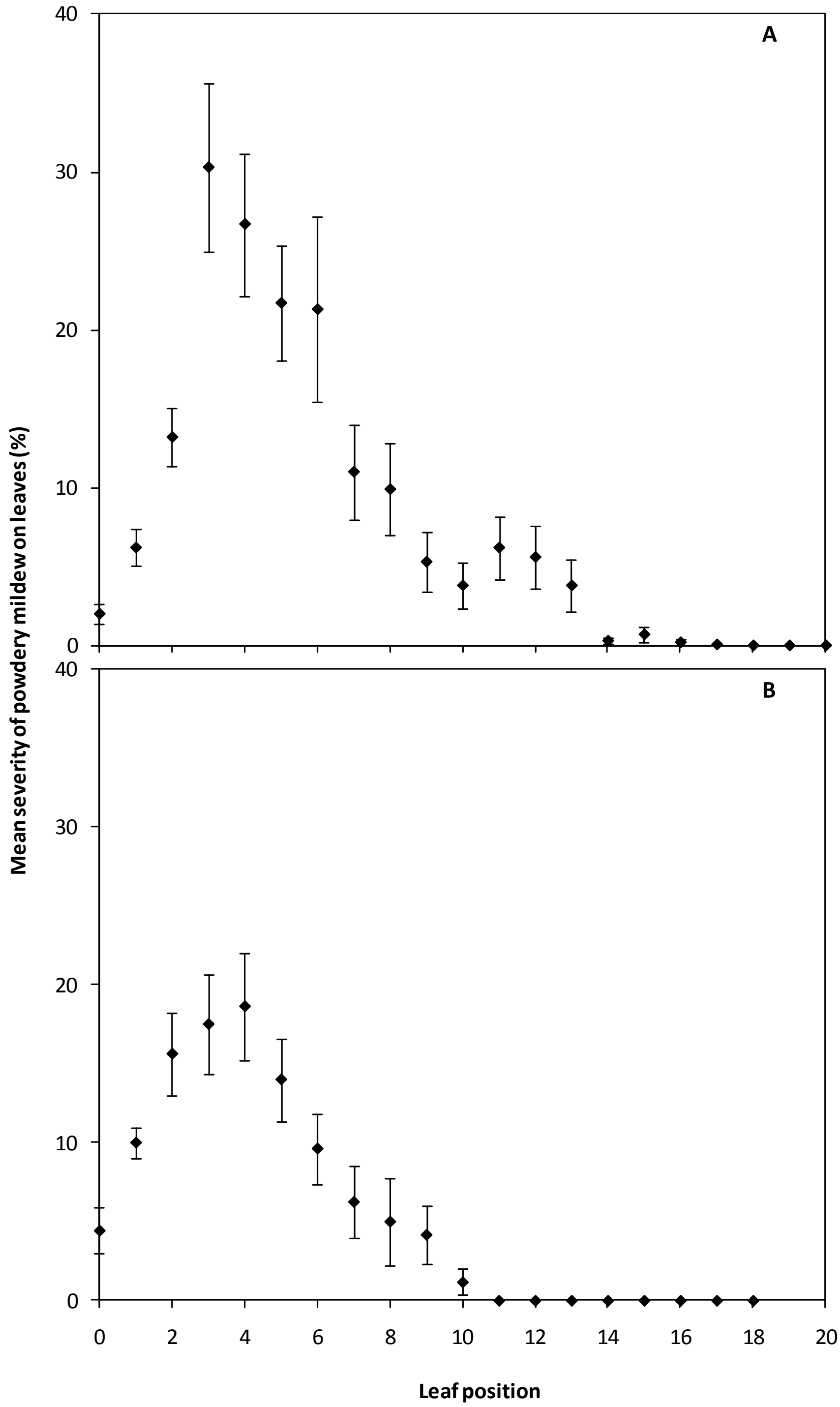

Figure 1.2. The effect of leaf position on shoots of glasshouse-grown Cabernet sauvignon vines on mean severity of powdery mildew 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Leaf position increases with increasing leaf maturity. Plants grown at an average of 25°C (A) or 18°C (B) prior to inoculation. The error bars represent standard error of the mean.

Table 1.3. The mean and standard deviation (sd) for leaf position of maximum powdery mildew severity for plants grown at an average of 18 or 25°C prior to inoculation, and the mean and sd for leaf position of maximum percentage of germinated *E. necator* conidia with secondary hyphae for plants grown at an average of 25°C prior to inoculation. Means within columns for maximum disease severity are not significantly different at *P* = 0.05.

	Leaf position				
	Maximum disease severity		Maximum percentage of conidia with secondary hyphae		
Average pre-inoculation temperature (°C)	Mean	sd	Mean	sd	*t* statistic ($P > 0.05$)
18	3.7	1.8	-	-	-
25	4.4	1.7	4.2	2.5	0.25

Bayesian model for powdery mildew development according to leaf position on a grapevine shoot

The overall Bayesian model was a good fit for the data for all plants (Figure 1.3). The means of parameters for posterior distributions for both the pathogen growth and leaf resistance models were examined to see if they varied according to pre-inoculation environment (Table 1.4). The magnitude of initial colonisation and the rate of colonisation, indicated by β_j and γ_j respectively in the pathogen growth model, were both greater on vines with a faster rate of leaf emergence before inoculation (Table 1.5). The probability that the overall posterior mean of β_j and γ_j for vines with a lower rate of leaf emergence was less than the overall posterior mean for the near optimum environment was 1.0 and 0.98, respectively.

For the leaf resistance parameters δ_j and ε_j, the probabilities that the overall posterior mean at the slower rates of leaf emergence were less than the overall posterior mean at the faster rate were 0.117 and 0.287, respectively. There was considerable variation among plants for individual posterior means for δ_j, including overlap between the two pre-inoculation environments (Table 1.4).

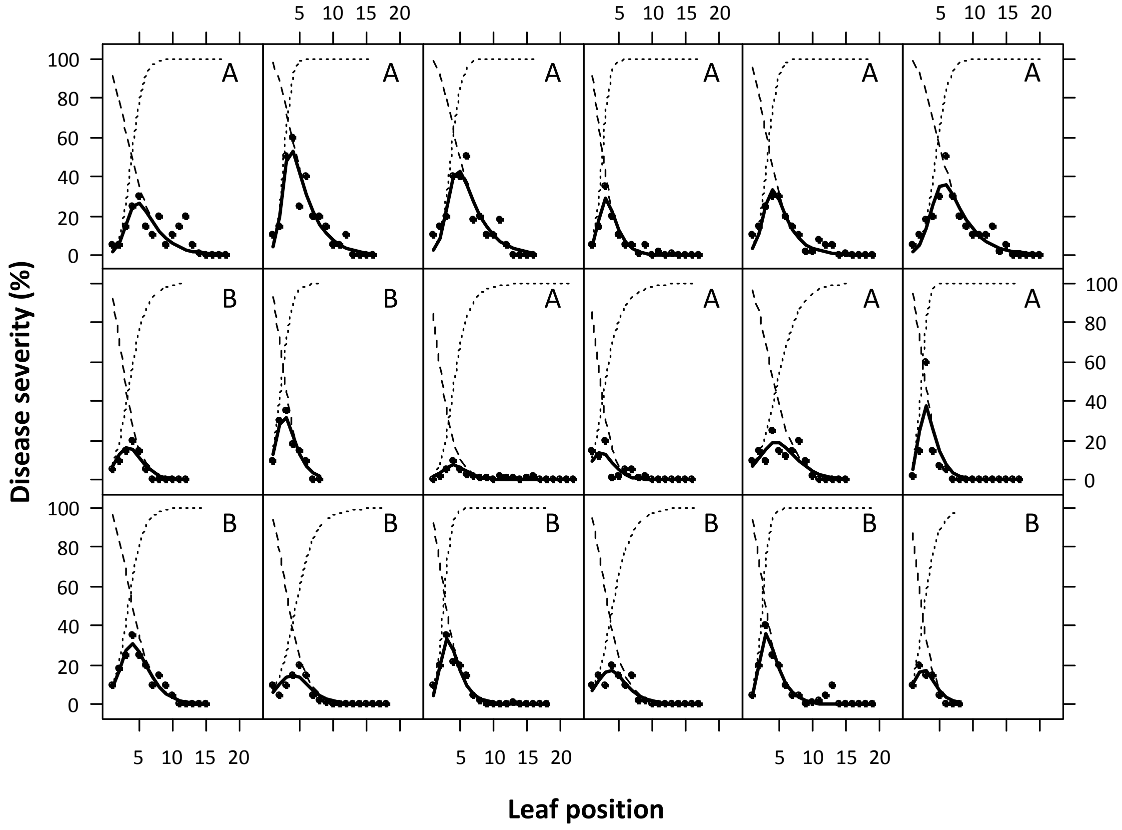

Figure 1.3. The effect of leaf position on shoots of glasshouse-grown Cabernet sauvignon vines on severity of powdery mildew 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Leaf position was correlated positively to leaf maturity. Each inset represents a single shoot and the observed data are shown as circles (•). The fitted curves are shown for the overall model (–), leaf resistance model (- - -) and pathogen growth model (···). Plants were grown at an average of 25°C (A) or 18°C (B) prior to inoculation.

Table 1.4. Means and standard deviations (sd) for 'posterior' distributions of the parameters β_j, γ_j, ε_j, and δ_j (refer to Table 1.1) for each plant and pre-inoculation temperature. Plants grown at an average of 18°C (plants 1-8) or 25°C (plants 9-18) prior to inoculation.

	β_j		γ_j		ε_j		δ_j	
Plant	Mean	sd	Mean	sd	Mean	sd	Mean	sd
1	3.30	*0.38*	0.99	*0.20*	4.41	*2.14*	0.47	*0.07*
2	3.41	*0.40*	0.78	*0.19*	4.38	*2.32*	0.54	*0.08*
3	3.15	*0.37*	1.06	*0.38*	4.11	*2.26*	0.80	*0.13*
4	3.32	*0.38*	0.81	*0.20*	4.38	*2.19*	0.55	*0.08*
5	3.25	*0.34*	0.68	*0.16*	4.32	*2.18*	0.44	*0.06*
6	3.21	*0.36*	1.18	*0.34*	4.16	*2.22*	0.77	*0.12*
7	3.43	*0.39*	0.97	*0.24*	4.36	*2.31*	0.64	*0.09*
8	3.26	*0.37*	1.44	*0.27*	4.46	*2.13*	0.67	*0.10*
9	5.10	*0.51*	1.24	*0.34*	3.13	*1.25*	0.68	*0.12*
10	4.85	*0.48*	1.94	*0.27*	3.44	*1.01*	0.58	*0.07*
11	4.94	*0.47*	2.01	*0.26*	3.60	*1.10*	0.58	*0.07*
12	5.14	*0.53*	2.23	*0.25*	4.44	*1.64*	0.67	*0.07*
13	5.03	*0.49*	1.31	*0.23*	2.80	*1.32*	0.37	*0.08*
14	5.03	*0.46*	1.90	*0.20*	3.53	*0.83*	0.38	*0.04*
15	4.98	*0.49*	1.38	*0.17*	3.88	*1.17*	0.36	*0.04*
16	5.00	*0.47*	2.00	*0.26*	3.57	*1.22*	0.65	*0.08*
17	4.89	*0.48*	1.51	*0.21*	3.48	*1.16*	0.45	*0.05*
18	5.02	*0.46*	1.12	*0.13*	3.85	*1.12*	0.33	*0.03*

Table 1.5. Overall means and standard deviations (sd) for 'posterior' distributions of the parameters β_j, γ_j, ε_j, and δ_j for each pre-inoculation temperature.

Average pre-inoculation	β^*		γ^*		ε^*		δ^*	
temperature (°C)	Means	sd	Means	sd	Means	sd	Means	sd
18	3.29	*0.30*	0.96	*0.24*	4.30	*2.08*	0.61	*0.09*
25	5.00	*0.41*	1.65	*0.22*	3.52	*0.87*	0.49	*0.07*

Early colonisation by *E. necator* in relation to leaf position

As leaves aged beyond leaf position 3, the percentage of conidia germinating to form a primary germ tube with or without secondary hyphae, generally decreased (Figure 1.4). A similar response was observed for the percentage of conidia germinating to form secondary hyphae (Figure 1.6). In contrast, the response for the percentage of conidia with primary germ tubes, but no secondary hyphae, was relatively flat, although there was a downward trend as leaves matured (Figure 1.5). The mean modal leaf position for the maximum percentage of conidia germinating to form secondary hyphae was 4.2, which was not significantly different ($P = 0.6$) to the mean modal leaf position with maximum powdery mildew severity which was 4.4 (Table 1.3). Powdery mildew was not observed macroscopically on leaves at position 17 (Figure 1.1A), whereas conidia with secondary hyphae were observed on leaves at position 17 (Figure 1.6), indicating there may have been a level of colonisation not visible to the naked eye, relative to younger leaves.

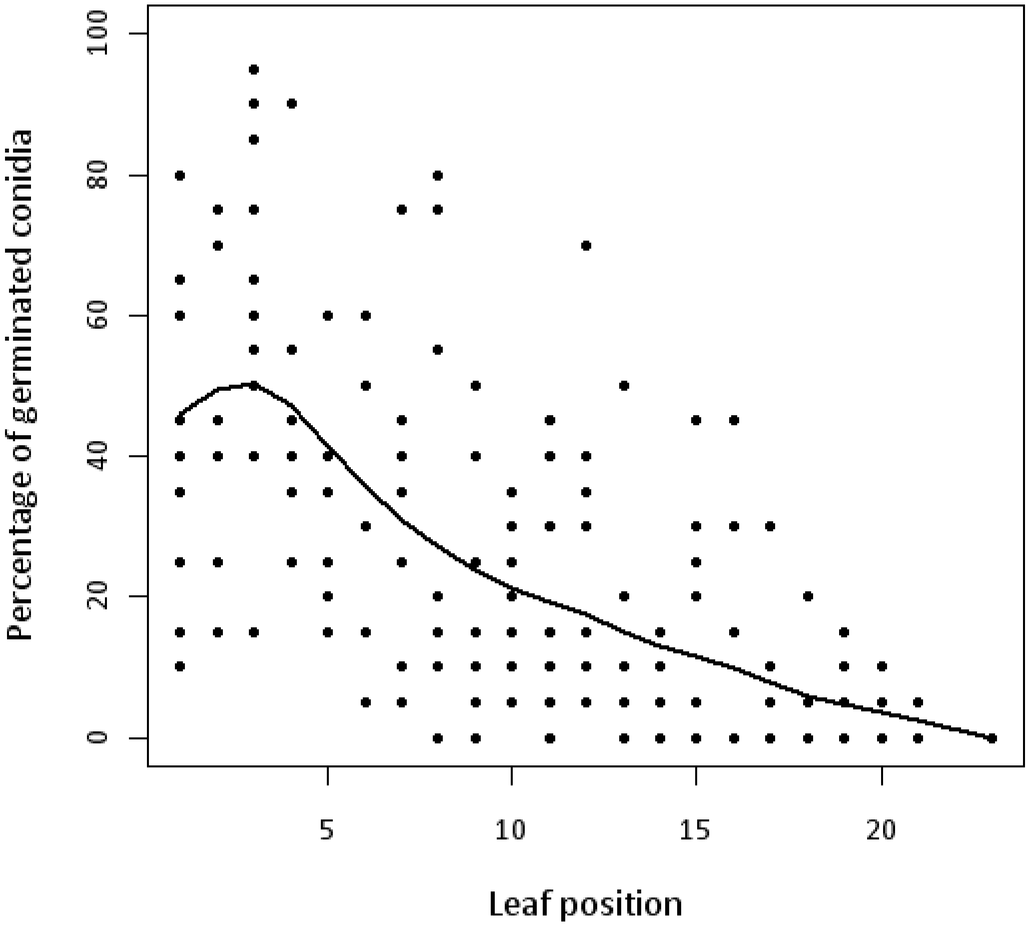

Figure 1.4. The effect of leaf position on shoots of Cabernet sauvignon vines, grown at 25^{o}C in the glasshouse, on percentage germination of *E. necator* conidia (n=40), 72 h after inoculation. The adaxial surface of each leaf was inoculated by transferring conidia with a paint brush from 12 day-old cultures. Leaf position was correlated positively to leaf maturity and all leaves were tested at the same time.

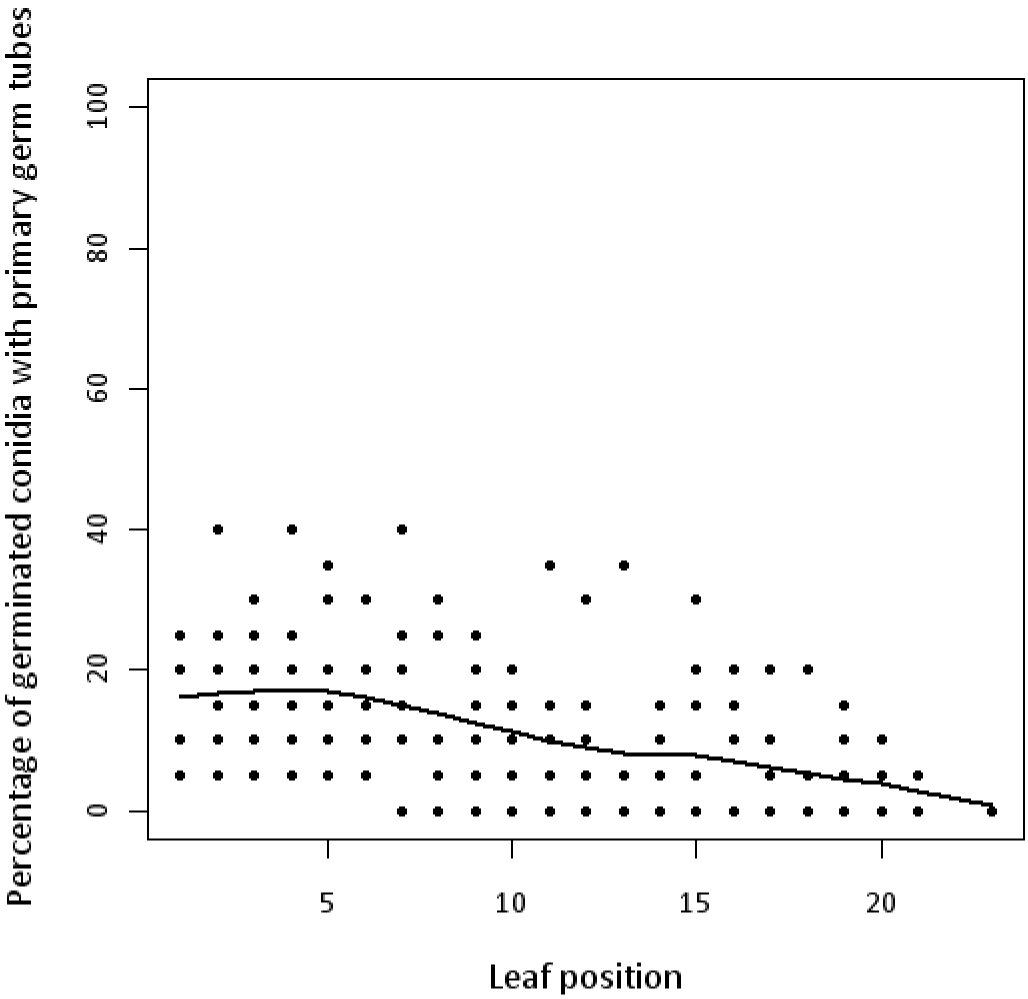

Figure 1.5. The effect of leaf position on shoots of Cabernet sauvignon vines, grown at 25^{o}C in the glasshouse, on the percentage of germinated *E. necator* conidia with primary germ tubes but no secondary hyphae (n=40), 72 h after inoculation. The adaxial surface of each leaf was inoculated by transferring conidia with a paint brush from 12 day-old cultures. Leaf position was correlated positively to leaf maturity and all leaves were tested at the same time.

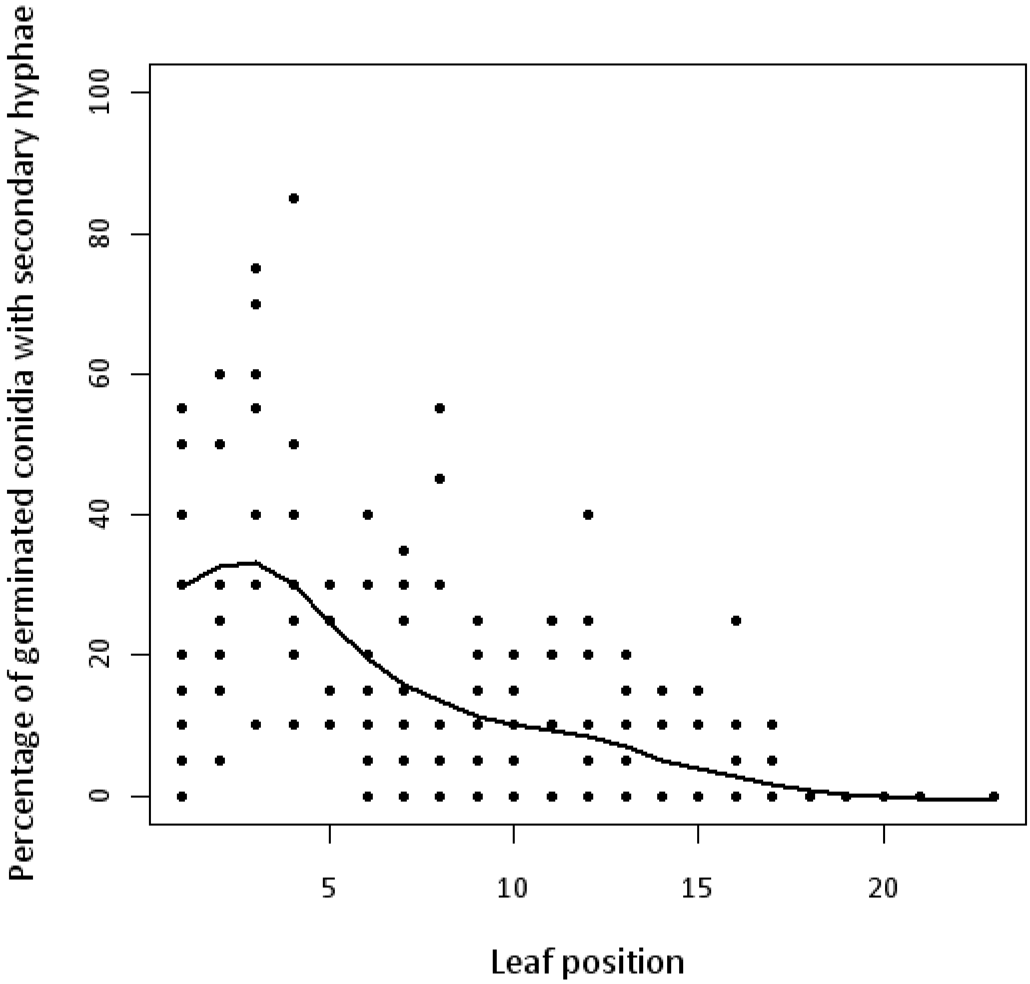

Figure 1.6. The effect of leaf position on shoots of Cabernet sauvignon vines, grown at 25^{o}C in the glasshouse, on the percentage of germinated *E. necator* conidia with secondary hyphae (n=40), 72 h after inoculation. The adaxial surface of each leaf was inoculated by transferring conidia with a paint brush from 12 day-old cultures. Leaf position was correlated positively to leaf maturity and all leaves were tested at the same time.

DISCUSSION

This study demonstrated that the colonisation of Cabernet sauvignon leaves by *E. necator* declined as leaves aged after expansion, as reported previously (Doster and Schnathorst 1985, Singh and Munshi 1993). The penetration of epidermal cells by germinating *E. necator* conidia declined as leaves matured, suggesting expression of leaf ontogenic resistance. Unlike previous studies, Bayesian analysis described how disease severity initially increased and then decreased due to leaf ontogenic resistance. Differences in the pre-inoculation environment affected the incidence and mean severity of powdery mildew on leaves, with a higher rate of leaf emergence leading to a greater proportion of diseased leaves per shoot and higher severity per leaf position.

The results clearly indicate a peak in leaf susceptibility to disease according to leaf position. However, the severity of powdery mildew observed on immature and expanding leaves may be influenced by leaf expansion after inoculation. That is, after fungal penetration, the leaf continues to expand resulting in an apparent reduction in area colonised epiphytically by *E. necator* if the rate of leaf expansion is greater than the rate of fungal colonisation. When compared with the use of detached leaves for inoculation studies, rapid leaf expansion after inoculation may have resulted in an apparently lower severity per area of leaf when compared with leaves with slower or completed expansion. Nevertheless, the response of early pathogen colonisation to increasing leaf position (Figures 1.4 and 1.6) was similar to the response observed for disease severity (Figure 1.2A&B). The percentage of conidia that developed secondary hyphae was a response variable that was not influenced by leaf expansion. Similarly, Reuveni (1998) used a detached grapevine leaf disc assay, where there was no expansion in leaf area, to assess the severity of downy mildew, another biotrophic pathogen, and reported a response curve to increasing leaf position that was similar to that reported here for powdery mildew.

The development of secondary hyphae of *E. necator* was severely inhibited as leaves of Cabernet sauvignon vines aged. In grape berries, ontogenic resistance did not reduce conidial attachment, germination or appressorium formation of *E. necator* (Ficke *et al*. 2003). However, grape berries that were ontogenically resistant prevented penetration by *E. necator*, although cuticle and cell wall thickness did not appear to be the principal mechanism of resistance (Ficke *et al*. 2004). The studies of Ficke *et al*. (2004) suggest that the likely mechanism might involve biochemical or ultrastructural modification of the cuticle or cell wall in resistant berries. In contrast to the study of Ficke *et al*. (2003), there appeared to be a linear but slight decline in the percentage of conidia with primary germ tubes but no secondary hyphae (Figure 1.5). Therefore, pathogen development might have been stopped before and after appressorium formation during the pre-penetration phase of pathogen development. Hence, the mechanisms that effect ontogenic resistance in grape leaves might be different to those in grape berries.

There have been limited studies on the effects of non-extreme pre-infection temperatures during plant growth on the ability of a pathogen to colonise a target plant organ. Rubio-Covarrubias *et al*. (2006) found that the penetration frequency of *Phytophthora infestans* zoospores in potato tubers was lower when plants were preconditioned at 16°C rather than at 24°C. In this study, Bayesian analysis indicated there was no significant difference between plants grown in the two different environments for the parameters of the leaf resistance model, yet there was a significant difference for the pathogen growth model. One interpretation of this result is that pathogen colonization was influenced by the nutritional, physical or some other quality of leaf tissue generated in the two different environments, whereas mechanisms that suppressed pathogen penetration and colonisation were expressed to a similar extent in leaves of equivalent quality, regardless of the environment under which they developed. While evidence for these hypotheses is lacking, biological aging is controlled intrinsically: cell contents change, cell walls thicken and transport and storage functions change (Shigo 1984). Presumably, specific but unknown mechanisms of ontogenic resistance are related to these changes and interact with the environment and how quickly leaves develop.

Colonisation of a plant by a biotrophic pathogen can influence the partitioning of resources for both plant and pathogen growth (Heath 2002) and for defence against pathogen invasion (Durrant and Dong 2004). How resources such as carbon, nutrients, water and light are allocated to plant growth, maintenance, storage, reproduction and defence can be influenced by the temperature under which plants are grown. This allocation is predicted by the hypotheses of carbon-nutrient balance (CNB; Bryant *et al*. 1983; Bazzaz *et al*. 1987; Tuomi *et al*. 1984) and of growth-differentiation balance (GDB; Lorio 1986; Herms and Mattson 1992). According to these hypotheses the accumulation of carbohydrates over levels needed for growth can be used for carbon-based secondary metabolism, for example defence responses, and/or storage of carbohydrates in various forms (Bryant *et al*. 1983; Tuomi *et al*. 1984). The mean size of fully expanded leaves immediately before inoculation, measured as lamina length, did not change in response to pre-inoculation environment, but the measurements taken did not provide for an estimate of the rate of leaf growth. More rapid plant growth at the higher pre-inoculation temperature may have resulted in greater losses of carbon via maintenance respiration (Amthor 1984) and, consequently, the metabolism of secondary metabolites for defence would be retarded. Additionally, leaves taking a longer time to develop, during slower growth at the lower pre-inoculation temperature, may have formed a thicker cuticle than leaves at an equivalent position on shoots developing at a faster rate. Leaf cuticle thickness can be an important part of resistance when the pathogen enters its host by direct penetration (Agrios 2005), such as *E. necator.* Heintz and Blaich (1989) found a negative linear relationship between cuticle thickness of young grape leaves and the intensity of *E. necator* sporulation on these leaves.

The expression of powdery mildew was clearly different in plants exposed to different pre-inoculation environments. Either the environment had a direct effect on some quality of the plant tissue to be colonised by *E. necator* and/or there was a direct effect of the environment, perhaps temperature, on the expression of pre-formed and/or induced mechanisms of resistance. Interpretation of the Bayesian analyses tends to support the former hypothesis more so than the latter, which in turn was dependent on the assumptions underlying the model and its validity.

CHAPTER 2 – MAXIMUM POWDERY MILDEW INFECTION IN LEAVES COINCIDES WITH THE SINK TO SOURCE TRANSITION

INTRODUCTION

Plant pathogens display varying levels of host and tissue specificity (Milgroom and Peever, 2003). In Chapter 1, leaf ontogenic resistance to *E. necator* was quantified for grapevine shoots developing in two different environments prior to inoculation. Variation in ontogenic resistance among leaves on a shoot was not related linearly with leaf position but rather there was a peak in susceptibility to infection by *E. necator* as leaves were expanding. Powdery mildew fungi derive their nutrition from living host cells and an understanding of the carbohydrate status of the growing leaf in relation to its susceptibility to infection by *E. necator* should aid definition of leaf characters associated with ontogenic resistance.

In the very early stages of growth, the leaf depends on inorganic and organic nutrients supplied from the export of nutrients from older leaves. As the leaf grows, an increasingly greater proportion of mineral nutrition comes directly via the roots (Milthorpe and Moorby 1969) and carbon continues to be supplied from older leaves (Turgeon and Webb 1973). Before the leaf is fully expanded, it becomes a net exporter of carbon and mineral elements (Ho and Shaw 1979), although concurrent import and export of nitrogen, phosphorus and potassium proceeds over a large part of its development (Dale and Milthorpe 1983). Thus, during its ontogeny, a leaf is converted from a net importer (sink) to a net exporter (source) for assimilates. There is a period of time when a leaf is importing and exporting concurrently, although not necessarily from the same part of the leaf (Turgeon 1973). This physiological maturation usually occurs from the tip to the base of the leaf blade in a function known as the photosynthetic sink to source transition (Turgeon and Webb 1975). The timing of the transition from leaf photosynthetic sink to source is correlated with attainment of a positive carbon balance: import stops and export begins when the supply of assimilates exceeds the growth and respiratory needs of the leaf (Pate and Atkins 1983). Coleman (1986) suggested that leaves are most susceptible to biotic and abiotic stress when making this transition from photosynthetic sink to source, on the basis that they may be nutritionally adequate for pests but not chemically or structurally well defended.

Leaves of *Vitis vinifera* begin exporting their photosynthates when they are 30-50% of their final area (Hale and Weaver 1962; Yang and Hori 1980), and importation ceases when leaves are 50-75% of their final area (Hale and Weaver 1962; Koblet 1977). When this occurs may depend on the cultivar (Yang and Hori 1980). Thus, percentage of final leaf size might be used as a descriptor/predictor of leaf status with regard to photosynthetic sink to source transition (Hale and Weaver 1962; Yang and Hori 1980).

Destructive sampling to estimate stage of expansion is a common procedure during studies of leaf physiology or host-pathogen interactions but creates some experimental difficulties. Removal of leaves before they have reached their final area results in the final area not being able to be measured directly for individual leaves and also impacts on other leaves on the developing shoot by removing a source or sink leaf. In grapevine, the width and length of the lamina or lamina length alone is highly correlated to leaf area (Elsner and Jubb 1988; Schultz 1992). Therefore, the area of a particular leaf can be estimated from its lamina length using a cultivar-specific model. However, to predict the final size of a particular leaf requires a model to describe lamina length as function of time during leaf expansion, with the final (predicted) lamina length used to estimate final leaf area, using the relationship between lamina length and leaf area.

The aim of this research was to test the hypothesis that maximum severity of powdery mildew occurs when grapevine leaves are infected by *E. necator* during the photosynthetic sink to source transition. The second aim was to develop a method to rapidly identify which leaf on a shoot of Cabernet sauvignon is in the sink to source transition by using the observed lamina length as a percentage of predicted lamina length at maximum leaf expansion.

MATERIAL AND METHODS

Plant material

Plants of *V. vinifera* cv Cabernet sauvignon were propagated by the same method as described for the inoculation study (Chapter 1). Ten and eight additional plants were grown at average temperatures of 25^{o}C and 18^{o}C, respectively, at the same time and under the same conditions as for the experiment described in Chapter 1. When all plants developed approximately 20 nodes, 'source' leaves for carbohydrate were treated with $^{14}CO_2$, as described below, or all leaves were inoculated with *E. necator* as described in Chapter 1. Leaf positions for both the C^{14} and inoculation studies were were recorded using the same protocol as described in Chapter 1.

Radio-labelling and autoradiography procedure

In the glasshouse in which the plants were propagated, the two youngest fully expanded leaves on opposite sides of the stem of each shoot were enclosed in a polythene bag and sealed around the petiole. A stock solution containing 100 µg and 0.37 MBq of $NaH^{14}CO_3$ per ml was diluted with 1.19 mM $NaHCO_{3,}$ pH 9.5, to 14.8 kBq per ml. A total of 17.76 kBq $^{14}CO_2$ was then released inside the polythene bag by addition of 0.5 ml of 20% lactic acid (v/v) to 1.2 ml of diluted $NaH^{14}CO_{3.}$ Exposure of leaves to $^{14}CO_2$ was at 08:00 and photosynthesis was allowed to continue for 2 h before removal of the polythene bag. After 24 h, leaves exposed to $^{14}CO_2$ and all leaves distal to the exposed leaves were cut from the shoot and dried for 7 days in the dark in a plant press at room temperature. Dried leaves of each shoot were then exposed to general-purpose X-ray film (Agfa, UV-C) in a standard medical X-ray cassette (Ultra Vision^{TM}) for 3 weeks prior to film development. On each shoot, the leaf position for the sink-to-source transition was designated as the youngest leaf showing no visual evidence of ^{14}C accumulation after inspection of autoradiographs.

Relating lamina length to area

The tight correlation between lamina length and area, observed in other studies, was confirmed by harvesting all leaves from four plants prepared in each pre-treatment environment and measuring the lamina length and area of each leaf. Leaf area was measured using an electronic planimeter (Paton and CSIRO, Australia).

Data analyses

A Student's *t* test (Snedecor and Chochran 1989) was used to test the hypothesis that the means for lamina length of leaves expressing maximum powdery mildew severity, as determined in Chapter 1 and rounded to the nearest leaf position, and those for the sink-to-source transition were not significantly different from each other. The comparison was done separately for each pre-treatment environment.

The relationship between lamina length (x) and leaf area (y) was determined by the least-squares fit to $y = cx^b$, where c and b were constants calculated using the 'Power' trendline function in Microsoft Excel® 2007.

Prediction of final lamina length

Additional plants were grown for measuring lamina lengths for each leaf multiple times during expansion, until leaves were inoculated with *E. necator* or treated with radio-labelled carbon. Lamina lengths on all leaves of each shoot were measured once 4-5 leaves had emerged and then every 3–4 days until the day of treatment and inoculation. Data were generated for 1,111 leaves across the two pre-inoculation environments and these data were used by G. Lee (University of Tasmania) to model the temporal development of lamina length as described in Appendix 1. Briefly, the final lamina length (ϕ_1; mm) of an individual leaf, as a function of thermal time (t; degree days above 10°C), was estimated by using the mixed effects model described by Equation 1:

$$Y_{ijk} = [\phi_{1ij}/(1 + \exp(-(t_{ijk} - \phi_{2ij})/\phi_{3ij}))] + \varepsilon_{ijk} \qquad (1)$$

$$\phi = [\phi_{1ij}, \phi_{2ij}, \phi_{3ij}] = [\beta_1, \beta_2, \beta_3] + [b_{1i}, b_{2i}, b_{3i}] + [b_{1ij}, b_{2ij}, b_{3ij}] = \beta + b_i + b_{ij}$$

where Y_{ijk} are repeated measures (k = 1,2,…,K_{ij}) of lamina length of individual leaves (j = 1,2,…,J_i) on individual vines (i = 1,2,…,I) as a function of thermal time (t). The final lamina length (ϕ_1) is as $t \rightarrow \infty$. The inflection point of the curve (ϕ_2) is the value of t at which the response attains $\phi_1/2$ and the scale parameter (ϕ_3) represents the distance on the abscissa between ϕ_2 and the point where the response attains $\phi_1/(1 + e^{-1}) \sim 0.73\ \phi_1$. The fixed effects β_1, β_2 and β_3 are the population means for final lamina length, inflection point and the distance on the abscissa, respectively. The random effects are normally distributed at the vine level, $b_i \sim N(0,\Psi_{vine})$, and leaf level, $b_{ij} \sim N(0,\Psi_{leaf})$, and are assumed to be independent for different vines, and leaves within vines. The within group errors are normally distributed as $\varepsilon_{ijk} \sim N(0,\sigma^2)$ and are assumed to be independent for different i, j and random effects.

Random effects were tested by a likelihood ratio test (Pinheiro and Bates 2000), to determine whether or not final lamina length varied significantly among vines and among leaves on vines for each parameter. Random effects were included in the final model at two (nested) levels. The random effects included in equation 1 were for the vine level, ϕ_{1i} and at the leaf within vine level, ϕ_{1ij} and ϕ_{2ij}.

Maximum lamina length was predicted for leaves identified as expressing maximum disease severity or transitioning from photosynthetic sink to source by using the lamina length on on the day of inoculation or treatment with $^{14}CO_2$ as an input to the model described in appendix 1. A *t* test was used to test the hypothesis that the lamina length as a percentage of predicted final lamina length of leaves expressing maximum powdery mildew severity was not significantly different from the mean for the sink-to-source transition. Data were arc-sine transformed (Milligan 1987) prior to conducting the *t* test.

Results

The sink to source transition in leaves of primary shoots of Cabernet sauvignon was clearly evident in the autoradiographs as shown in the typical example in Figure 2.1. The transition occurred, on average, at leaf positions 3.8 and 4.7 for plants grown at average temperatures of 18 and 25^{o}C, respectively (Table 2.1). The overall mean modal leaf position at maximum disease severity and the mean leaf position when leaves were in the transition from photosynthetic sink to source were similar for plants grown at similar average temperatures prior to inoculation or assay (Table 2.1). Different pre-treatment environments did not result in significant differences ($P > 0.05$) in the mean leaf position for each response variable listed in Table 2.1.

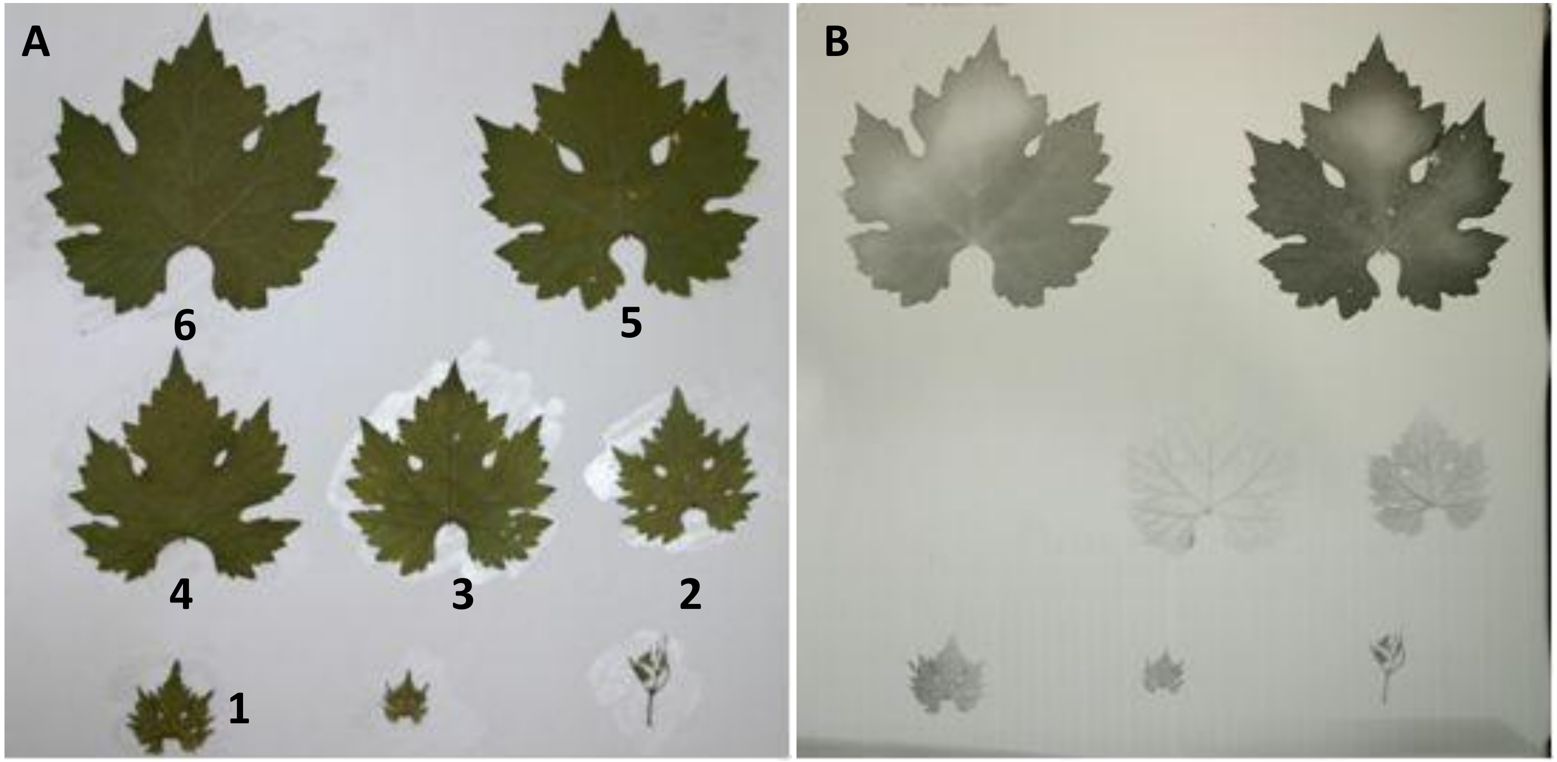

Figure 2.1. The fate of ^{14}C in a primary shoot of Cabernet sauvignon after exposure of two 'source' leaves (5 & 6) to $^{14}CO_2$. (A) Dried leaves numbered according to position from the apex, where the leaf at position 1 had a lamina length of ≥ 30 mm, and (B) corresponding autoradiograph showing that leaf 4 was the youngest leaf that had completed the sink to source transition because it had ceased importing ^{14}C. The vine was grown at an average of 25°C prior to assay.

Table 2.1. The mean and standard deviation (sd) for leaf position of maximum powdery mildew severity (data derived from Chapter 1) and of the leaf in the sink to source transition as determined by cessation of import. Data presented are for shoots grown in two different environments prior to inoculation or assay. Means within columns are not significantly different at *P* = 0.05.

Average pre-treatment temperature (^{o}C)	Leaf position			
	Maximum disease severity		Sink to source leaf	
	Mean [a]	sd	Mean	sd
18	3.7	1.8	3.8	0.8
25	4.4	1.7	4.7	1.1

[a]Mean of the modes

There was a significant relationship (P < 0.001 for the regression) between lamina length and leaf area (R^2 = 0.96, Figure 2.2), which justifies the use of lamina length as an indirect measure of leaf area. There was no significant difference (P > 0.05) in mean lamina length between leaves in the sink to source transition and leaves with maximum disease severity for either pre-treatment environment (Table 2.2).

Of the 36 leaves identified to be in the sink to source transition or expressing maximum disease severity, only 10 final lamina lengths were able to be predicted to approximately 6 mm accuracy. Estimates for the other 26 leaves were unreliable because there were too few observations (i.e. < four unique values) during leaf expansion. Completion of the sink to source transition occurred at a mean of 94 % (n = 4) predicted final lamina length and maximum disease severity occurred at a mean of 90 % (n =4) predicted final lamina length.

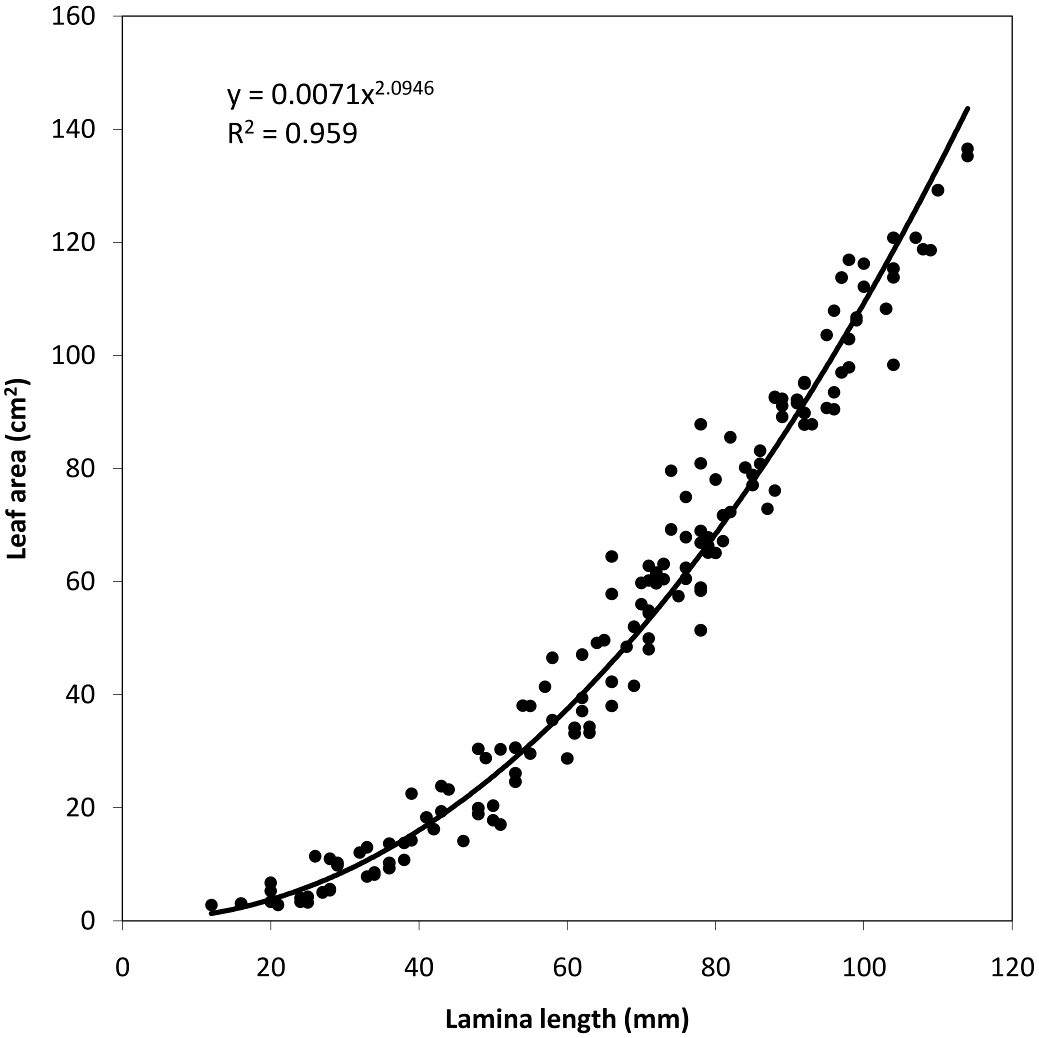

Figure 2.2. The relationship between lamina length and leaf area of Cabernet sauvignon leaves grown at an average temperature of 18 or 25°C.

Table 2.2. The mean and standard deviation (sd) of lamina length on the day powdery mildew severity was measured or the leaf detached for determination of the sink to source transition. Data presented for shoots grown in two different environments prior to inoculation or assay. Means within rows are not significantly different (*P* = 0.05).

Average pre-treatment temperature (°C)	Lamina length (mm): Maximum disease severity		Lamina length (mm): Sink to source leaf		
	Mean	sd	Mean	sd	*t* statistic ($P > 0.05$)
18	92	8.5	90	7.0	*0.37*
25	83	13.2	87	6.1	*0.80*

DISSCUSSION

There was a clear association between leaf position for maximum severity of powdery mildew and the position of the leaf completing the sink to source transition. Moreover, this correlation was maintained when plants were subjected to different conditions prior to inoculation or assay. Leaves of *V. vinifera* cv. Cabernet sauvignon were most susceptible to infection by the powdery mildew fungus immediately after leaves had ceased to import carbohydrate. At this stage of physiological development, export of assimilates begins as import ceases (Turgeon and Webb 1973) and there are many structural and metabolic changes associated with accumulation and export of photosynthate in the transition to a source organ (Dickson and Larson 1981). Along with the associated physiological changes, obvious morphological changes also occur, including thickening of the cuticle.

Before the sink to source transition, young leaves use carbohydrates efficiently to build cellular structures and produce energy (Masclaux *et al*. 2000). Newly unfolded leaves rely on other plant parts for their carbohydrate supply and concentrations of free sugars are low as they get used up immediately to build the new lamina. Host resistance to pathogen attack may be weak if the leaf is allocating more resources to growth, rather than defence. Hence, activation of secondary biochemical pathways required for induced defence could be constrained in immature leaves that are a strong sink for carbohydrate. The low severity of disease observed on newly unfolded leaves may be the result of a number of factors including rapid expansion of leaf area, a lack of secondary metabolism for defence and possibly a pathogen that is less able to compete with the sink strength of the host (Cole 1966).

When leaves are expanding and changing from a sink to a source they have two sources of photosynthate; namely, *in situ* and imported. The completion of the sink to source transition occurred when the leaf was nearly fully expanded, which also coincides with the maximum photosynthetic rate of the leaf, which declines thereafter (Kriedemann *et al*. 1970; Zufferey *et al*. 1999). When the rate of formation of sucrose exceeds its rate of removal by the transport system, photosynthate is diverted into starch synthesis (Mullins *et al*. 1992). The concentrations of starch, reducing and nonreducing sugars are influenced by leaf development. For instance, Kliewer (1966) found that the concentration of glucose and fructose in the leaves of field grown grapevines increased from unfolding until the individual leaf had expanded to one third of its final area, after which concentrations of these sugars reached a plateau. Schnee *et al.* (2010) found a similar relationship, with glucose concentration of grapevine leaves increasing when leaves were between 6 and 8 days old, after which a plateau was reached. However, Schnee *et al.* (2010) found the establishment of ontogenic resistance, as measured by sporulation intensity, correlated with the steep increase in cell glucose concentration. Their study was performed using leaf disks and so the results may not be comparable to assays where leaves are inoculated when attached to whole plants. Progressive depletion of other carbohydrates in older leaves, post the sink to source transition, may limit the quantity or quality of sugars required by biotrophic pathogens (Chiba 1966, Bell 1981 cited in Coleman 1986). Glucose has been found to be the major source of carbon transferred from cells of wheat plants to the powdery mildew pathogen *Blumeria graminis* f.sp. *tritici* (Sutton *et al*. 1999). Acid invertase, the enzyme responsible for hydrolysing sucrose to glucose and fructose, is usually most active in rapidly growing leaves (Wardlaw 1990). An ideal ecological niche for infection by *E. necator* might be created immediately after completion of the sink to source transition due to high amounts of the right form of available sugar, considering that the powdery mildew fungi are classed as high-sugar pathogens (Horsfall and Dimond 1957).

The use of lamina length to predict which leaf on a shoot of Cabernet sauvignon is transitioning from sink to source may be useful in studies where destructive sampling and autoradiography cannot be conducted. Shortcomings in the data collected for the mixed-effects model (Appendix 1) did not enable all target leaves to be predicted and this limited treatment comparisons. Modelling (Appendix 1) indicated that at least five measurements before destructive sampling were needed to obtain reliable estimates of maximum lamina length. Further work is also required to determine if the timing of the sink to source transition, in terms of the percentage of final leaf area as predicted by lamina length, varies according to cultivar (Yang and Hori 1980) and environmental conditions, including when the leaf emerges in the growth cycle of the grapevine. For instance, conditions which have been found to affect assimilate partitioning in plants include water availability (Lang and Thorpe 1986; Nandwal *et al*. 1996), light interception (Guan and Jane 1991; Pasumarty and Fountain 1993), and fruit load (Heuvelink 1997; Edson *et al*. 1993; Masahiko 2000). This potential variability of timing of when leaves make the transition from photosynthetic sink to source might make it difficult to make predictions of when a grapevine leaf of a particular cultivar is in the sink to source transition across the range of environments in which it is grown. However, it has been shown that grapevine leaves that are near full leaf area expansion are likely to be at their maximum susceptibility, and susceptibility declines thereafter. This means that as the grapevine shoot grows, only a small percentage of healthy foliage area is at maximum susceptibility to infection by *E. necator*.

Future studies investigating mechanisms of ontogenic resistance in relation to the photosynthetic sink to source transition may require greater spatial and temporal resolution of this process in leaves. Newer techniques such as image sequence analysis (e.g. Kummerlen *et al*. 1999) or chlorophyll fluorescence imaging (e.g. Siebke and Weis 1995) might replace the radiographic technique used here because the non-destructive analysis allows the study of dynamic responses, in both space and time, to environmental variables that influence carbohydrate metabolism and growth of leaves. These techniques, when combined with studies of host-pathogen interactions at the microscopic and molecular level, could provide new insights about host factors that determine infection efficiency and colonisation by *E. necator*. The sink to source transition in grapevine leaves is at least a physiological marker for high organ susceptibilty to powdery mildew. Whether maximum susceptibility of leaves associated with the sink to source transition of leaves is the consequence of the metabolic or developmental changes associated with the transition remains to be demonstrated. Therefore, physiological, morphological and molecular processes associated both with this transition and pathogen colonisation need to be deciphered.

CHAPTER 3 - PRIMARY SHOOT DEVELOPMENT IN CHARDONNAY AND PINOT NOIR

INTRODUCTION

In Chapter 1, it was demonstrated that the rate of leaf emergence affected the quality of grapevine foliage associated with leaf position and the incidence and severity of powdery mildew on all leaves of the shoot. In the vineyard, the rate of leaf emergence will also influence the amount of new and unprotected leaf tissue emerging since a fungicide application. Thus, quantification of the heterogeneity of grapevine foliage for relative susceptibility to *E. necator* infection should be implicit in the design of management strategies against powdery mildew. This heterogeneity has not previously been accounted for in calendar-based spray programs. For instance, strategies for managing powdery mildew in the Riverland region of Australia include recommendations for applying protective fungicides 2, 4 and 6 weeks after budburst. While this “2, 4, 6” rule accounts, in general, for the relatively rapid rates of leaf emergence after budburst in the region, it is not necessarily applicable in cooler regions as it does not take into account slower or more variable rates of leaf emergence. In the cool climate of Tasmania, there can be little or no apparent increase in leaf area during some very cool weeks in spring. Presumably residues of fungicides that decay slowly continue to provide an effective dose on susceptible leaves sprayed in previous weeks. Conversely, rapid leaf emergence and growth later in spring and early summer can result in a significant amount of leaf area that is unprotected by fungicide residue during a two week interval after a fungicide application. When seasonal conditions are this variable, rate of appearance of unprotected leaves might be an important factor in assessing the risk of powdery mildew, as it will influence the proportion of leaf area that is susceptible to infection by *E. necator* and potential for infection of the developing fruit.

The above-ground vegetative organs of grapevines consist of the trunk, arms, shoots and leaves. The number of buds and position on the trellis is determined by the pruning method used during winter dormancy in the particular vineyard. As noted earlier, most Tasmanian vineyards use some variant of 'cane pruning', with two or more one year old canes retained to give approximately 10 dormant buds per retained cane available for spring growth. Shoot growth from these buds begins in spring with budburst generally occurring in early September. Temperature is the dominant factor controlling budburst, which generally occurs when the daily mean temperature exceeds 10°C (Baldwin 1966; Winkler 1970; Williams *et al* 1985). In temperate climates, budburst is typically an asynchronous process along the cane as buds burst over a period spanning several weeks, with a few remaining dormant or 'blind' (Martin and Dunn 2000). Distal buds burst earlier than those closer to the trunk along an individual cane (Antcliff and Webster 1955; Antcliff *et al*. 1957; Baldwin 1966). Buds furthest from the trunk are regarded as more vigorous throughout the season, a phenomenon thought to be caused by a persistence of apical dominance through dormancy and into the following season (Martin and Dunn 2000). Pruning methods, such as the arched cane system, have been used in Sultana vines to modify the distribution of bud vigour along the cane, resulting in more bunches on the middle part of the cane and shorter shoots on the distal part of the cane (May *et al*. 1978).

The vine produces its organs by cell differentiation at the apical meristem followed by growth that involves cell division and expansion. Phenology is the time course of organ emergence, development and growth, as influenced by environmental factors (Leith 1974). While nutrients, light and water are required for growth, the rate of cell division is primarily influenced by ambient temperature (Watson 1952). Other factors, such as day length and endogenous hormones can influence the timing and nature of organ development (Garner and Allard 1920; Chailakhyan 1979). Under otherwise comparable conditions, the rate of production of grapevine leaves has been shown to depend on air temperature (Buttrose

1969), having been linked with various measures of accumulated heat (thermal time). The base, or threshold temperature in thermal time calculations for *Vitis vinifera* may vary and also depend on the growth stage being analysed (Jackson and Cherry 1988; Moncur *et al*. 1989; Oliveira 1998). For leaves, however, a base of 10°C is generally accepted (Schultz 1992).

The importance of temperature in determining the rate of biological processes has also been utilised for predicting generation times of plant pathogens and hence disease risk. Temperature-based models of powdery mildew risk, such as the Gubler-Thomas (GT) index developed in California (Gubler *et al*. 1999), are based on the effect of temperature on the pathogen generation time. The GT index was tested in Tasmania (Evans 2005) but proved unreliable at predicting disease risk in Tasmania's mild temperature conditions. Thus, while further development of pathogen-based models is required, a complementary approach would be to estimate disease risk based on the development of the host.

In Germany, protective fungicides for downy mildew are timed according to the amount of new leaf area since the last spray (Bleyer *et al* 2001), based on models that describe shoot development as a function of thermal time calculated as degree days (Schultz 1992). This knowledge can be combined with the duration of the protective activity of fungicides deposited on leaves, to account for both spatial and temporal variation in the location and efficacy of spray deposits (Bleyer and Huber 1995). Field trials have shown that several fungicides control downy mildew for 21 days after their application on leaves (Bleyer *et al* 2001; Huber *et al*. 2002). Similarly, effective residues of sulfur, applied at the current label rate of 600 g product/100 L water for powdery mildew control, are known to persist on grapevine leaves cv. Sultana for at least 50 days under dry conditions (Emmett *et al.* 2002). Hence spray intervals are not limited by the degradation of this common fungicide, but by the growth of the vine. In order to develop disease-risk models that account for host growth, shoot development must be defined for specific varieties, climatic conditions and vine management regimes.

In this study, shoot development was quantified as the rate of leaf appearance, shoot growth and leaf area development over time on primary shoots of two commonly grown grapevine cultivars in Tasmania, Chardonnay and Pinot noir. The effect of the position of vertically trained shoots on the arms of cane-pruned vines and, in the case of Pinot noir, the upward or downward orientation of shoots in a commonly used trellising system was also investigated.

MATERIALS AND METHODS

Study sites and canopy management

The cultural characteristics and timing of bud burst and canopy manipulations were recorded for six single vines in each of four commercial vineyard blocks in the Coal River Valley of southern Tasmania, Australia. A total of 24 vines were studied in two blocks of Chardonnay and two blocks of Pinot noir. The vines were sampled along a diagonal transect crossing six adjacent rows near the centre of each block, with one vine selected per row. Budburst was taken as the date when at least 50% of the buds had reached the visible green stage (modified E-L stage 5 as described by Coombe 1995). Details, including dates of budburst and relevant management activities for each block are given in Table 3.1

Table 3.1. Location of blocks, cultural characteristics, and dates of budburst, shoot positioning and hedging. A dash indicates that shoots were not positioned downwards, but allowed to grow upwards restrained only by foliage wires.

	Block and variety			
	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
Location of vineyard	42°48′ S, 147°25′ E	42°44′ S, 147°29′ E	42°45′ S, 147°25′ E	42°44′ S, 147°29′ E
Trellis system	VSP*	arched cane VSP*	divided canopy	divided canopy
Intra row spacing (m)	1.5	1.25	1.35	1.25
Inter row spacing (m)	2.1	2.5	2.4	2.5
Vines per hectare	3175	3200	3086	3200
Date of budburst				
2005-06 season	1st Sep	29th Sep	13th Sep	19th Sep
2006-07 season	7th Sep	11th Sep	7th Sep	19th Sep
Date of downward shoot positioning				
2005-06 season	-	-	10th Nov	24th Nov
2006-07 season	-	-	8th Dec	12th Dec
Date of hedging				
2005-06 season	5th Jan	2nd Feb	15th Jan	2nd Feb
2006-07 season	3rd Feb	9th Feb	12th Jan	9th Feb

*Vertical Shoot Positioning

Each vineyard block comprised cane-pruned vines with two arms, pruned as per commercial practices to a target bud number of 20 per vine (10 per arm). Shoots were positioned vertically on arms of Chardonnay vines horizontally trained at site A and the arms were arched at site B (Figure 3.1). Pinot noir canopies at both sites (Figure 3.1) were divided vertically, with shoots positioned vertically on the upper arm and the lower arm having shoots positioned downwards by tying them to a lower trellis wire at approximately E-L stage 18 (Coombe 1995), before flowering commenced.

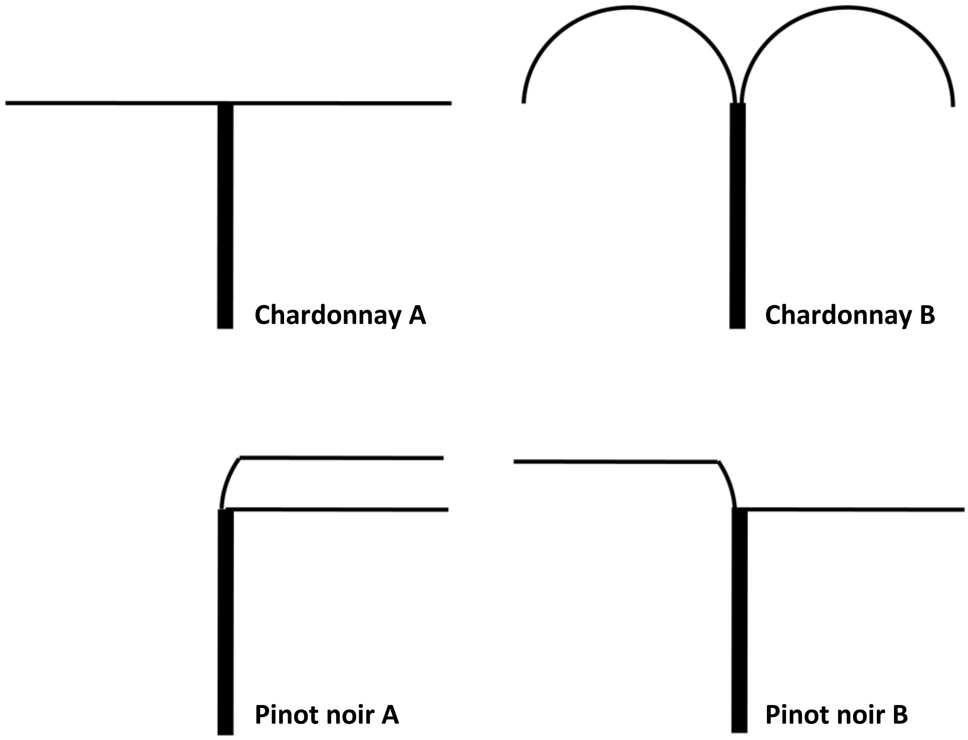

Figure 3.1. Schematic representation of the trellis system in different blocks: Trellis wires (not shown) run in the same plane as the page and thinner lines show dormant cane positions ('arms') following winter pruning. In Chardonnay A, new shoots are positioned vertically (VSP) on horizontal arms, with Chardonnay B having similar VSP on arched arms. In Pinot noir, A & B new shoots arising on the lower arms were positioned downwards, whilst those on the upper arm were allowed to grow upwards thus giving a horizontal split in the canopy.

Weather data

Daily minimum and maximum temperatures within the fruiting zone of the vine canopy at each site were measured using a FS-TH 'field station' (Avatel Inc., Fort Bragg, California) or Tinytag Plus (Gemini Data Loggers) pre-calibrated dataloggers with the sensor placed within a standard miniscreen, and programmed to record temperature every 30 min. Thermal time accumulated from bud burst for each site was calculated as growing degree days (GDD) above 10°C after Winkler (1970), according to equation 1.

$$GDD = \sum(T_{Dmax} + T_{Dmin})/2 + 10) \qquad (1)$$

where T_{Dmax} is the daily maximum air temperature (°C) and T_{Dmin} is the daily minimum air temperature (°C) recorded from the dataloggers. Mean monthly temperature and total rainfall per month during spring and early summer of each year of the study were obtained from the Australian Bureau of Meteorology (BOM) station at Hobart Airport (42°50' S, 147°30' E), which was the closest weather station to the study sites in the Coal River Valley. Long term averages for this station were based on data from 1958-2007.

Leaf emergence and shoot growth

Measurements of variables related to primary shoot growth were made in 2005-06 and 2006-07 for each of the six vines sampled per study site. On each arm, three buds that were distal, medial or proximal to the vine trunk were selected. Starting from when at least four leaves had appeared on shoots arising from these buds, lamina lengths of all leaves on the primary shoot and shoot lengths were measured at irregular intervals from 5 to a maximum of 21 days. Measurements continued until shoot tip pruning (hedging) was carried out in the commercial management regimes of each vineyard (Table 3.1).

The plastochron index (PI) is a method for measuring shoot development on a physiological rather than a temporal scale. In the present study it was calculated as a continuous function of time by logarithmic interpolation between the lamina lengths of the two apical leaves at either side of a reference length (equation 2) after the method of Erickson and Michelini, (1957);

$$PI = n + (\log L_n - \log 30)/(\log L_n - \log L_{n+1}) \qquad (2)$$

where 30 is the reference length in mm (Freeman and Kliewer, 1984; Schultz 1992), L_n is the length of leaf n and n is the number of the leaf equal to or just longer than the reference lamina length of 30 mm.

Leaf area estimation

To estimate leaf area, at least 150 leaves at each block were sampled from additional vines Lamina length of each leaf was measured along with leaf area, measured using an electronic planimeter (Paton and CSIRO, Australia). The relationship between leaf area (y) and lamina length (x) was determined by the least-squares fit to $y = cx^b$, where c and b were constants calculated using the 'Power' trendline function in Microsoft Excel® 2007. Constants are presented in Table 3.2.

Table 3.2. Parameter values and coefficients for leaf area calculations

Site	c	b	R^2
Chardonnay A	0.4663	2.1682	0.9940
Chardonnay B	0.5127	2.1259	0.9947
Pinot noir C	0.4381	2.1929	0.9937
Pinot noir D	0.4608	2.1856	0.9879

Data analyses

Leaf emergence as a function of calendar day

Mean plastochron development rate, calculated by dividing the plastochron index by the day after budburst, was plotted against calendar day for each season and site.

Leaf emergence, shoot growth and leaf area as a function of thermal time

The rate of leaf emergence, the rate of shoot growth or the rate of leaf area development for each shoot was estimated as the slope of the corresponding linear regression of PI, shoot length (cm) or leaf area (mm^2) against cumulative thermal time (degree days). Growth was near linear in the measurement period (four leaves emerged to commercial hedging) and rates were estimated as the slope of the corresponding linear regression against cumulative thermal time measured in degree days. Experimental designs were a 2 growing seasons x 3 bud position factorial for Chardonnay and a 2 growing seasons x 3 bud position x 2 shoot orientation factorial for Pinot noir, with vines in completely random designs with six replicates. 'Bud position' refers to the site of new shoot growth arising from the overwintering cane in the cane pruning system. Separate analyses of variance (ANOVA) for the estimated growth rates were carried out for each site and variety. For these analyses, ANOVA was calculated using GenStat 11.0 (VSN International Ltd). When F tests were significant at $P < 0.05$, the least significant difference (LSD) (Steele and Torrie 1981) was calculated at $P = 0.05$ for testing differences between mean growth rates. Shoots which failed to develop beyond seven nodes due to damage were excluded and treated as missing plots in the ANOVA.

RESULTS

Seasonal conditions

Overall, the 2006-07 season was cooler than 2005-06. With the exception of November in 2006-07, mean monthly maximum temperatures for September to January in both years were higher than the long term average (Figure 3.2). There was a greater heat summation in the 2006-07 season for the first 50 days after budburst, with the two Chardonnay sites showing the greatest difference (Figure 3.3). After approximately 50 days from budburst onwards, the situation reversed with a greater heat summation in 2005-06 than in 2006-07 at all sites except site A for Chardonnay where heat summation was similar for both seasons (Figure 3.3).

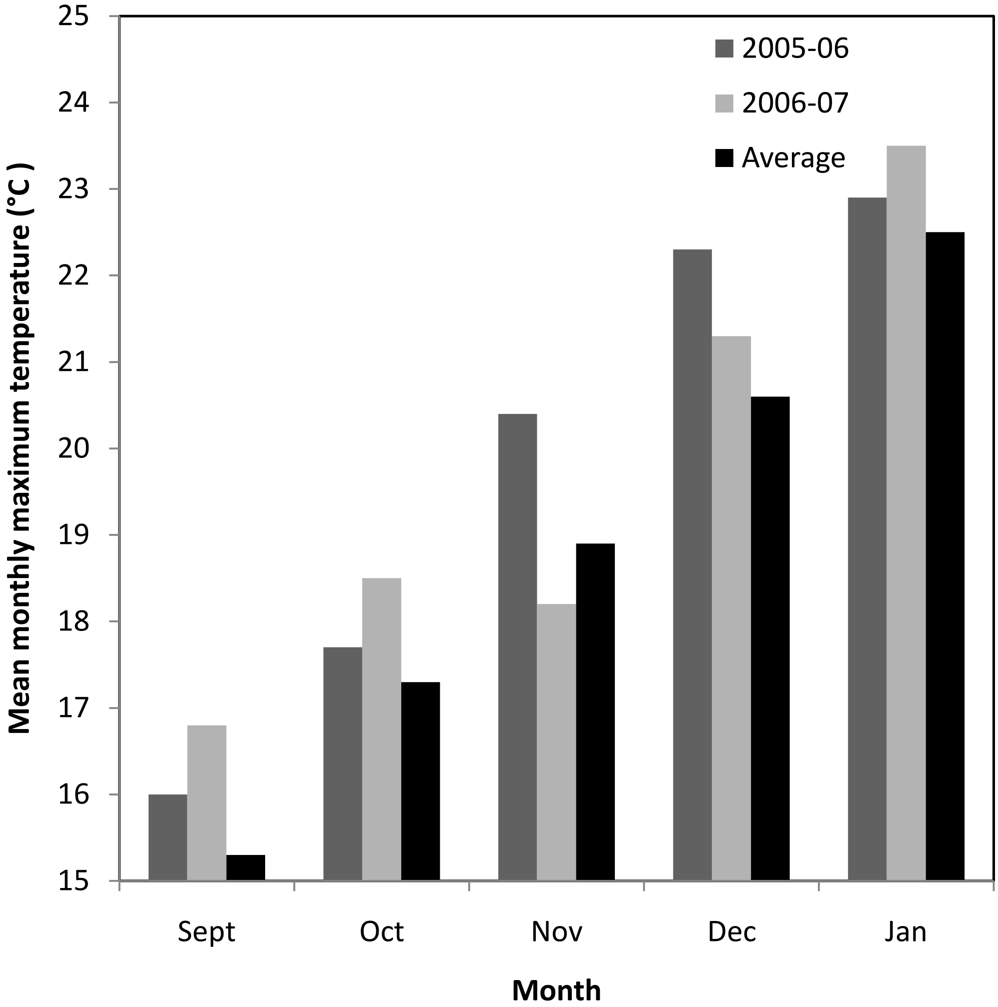

Figure 3.2. Mean monthly maximum temperature measured at Hobart Airport (42°50' S, 147°30' E) during the study period for 2005-06 and 2006-07 relative to the long term average based on 1958 - 2007 (www.bom.gov.au).

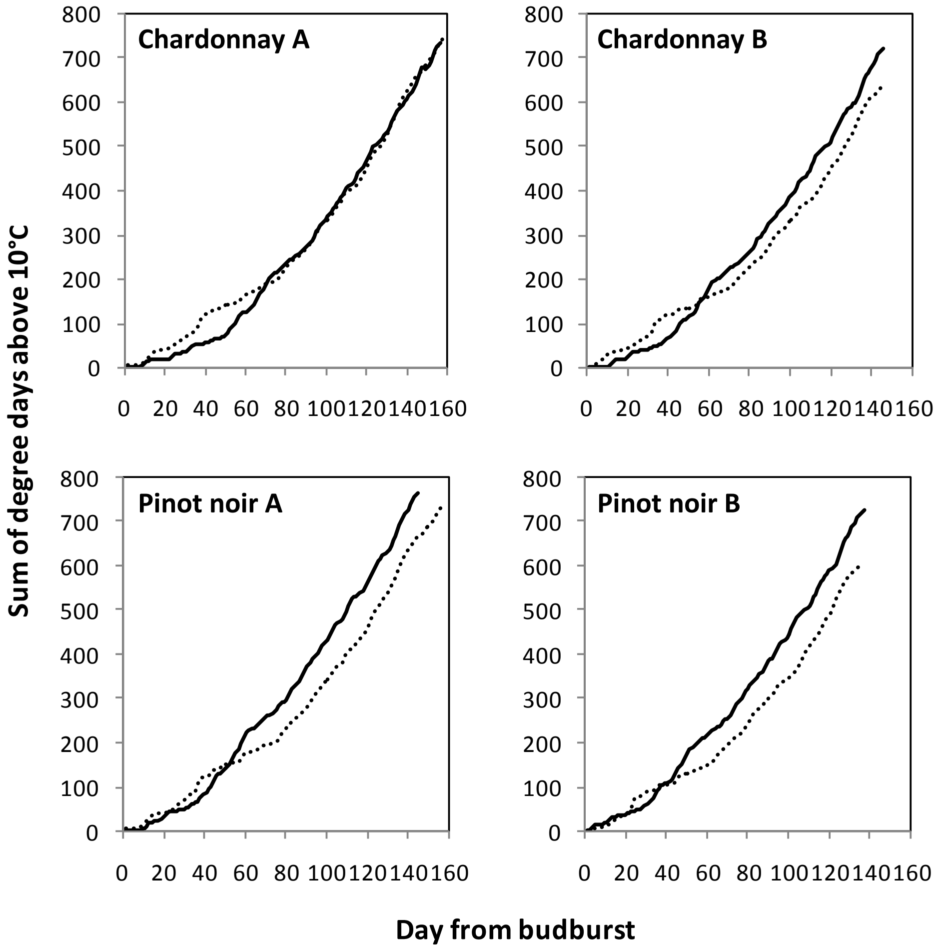

Figure 3.3. Accumulated degree days above 10°C, from budburst until hedging, for the sites shown during 2005-06 (–) and 2006-07 (···).

The 2005-06 season was unusually wet during spring and early summer, with above average rainfall from September to December (Figure 3.4). In contrast, 2006-07 season was generally drier than average in these months (Figure 3.4).

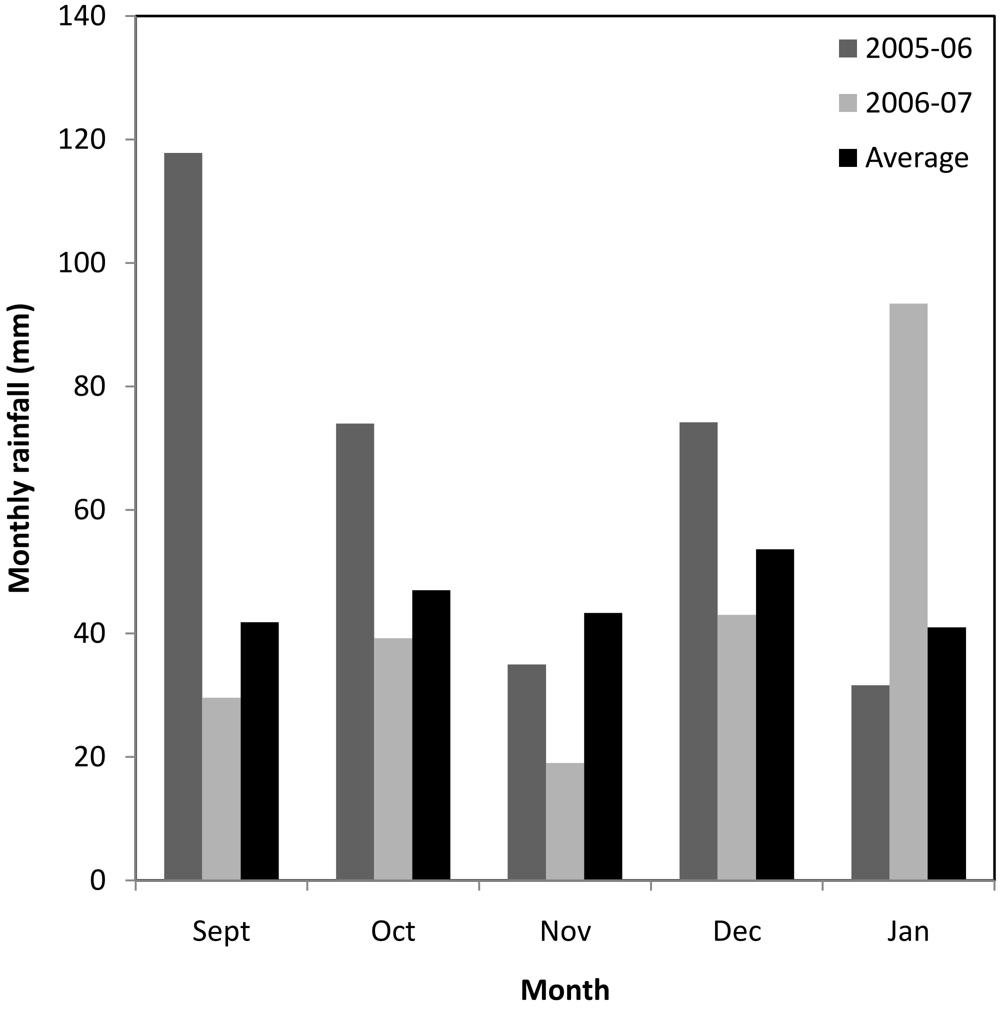

Figure 3.4. Monthly rainfall at Hobart Airport (42°50' S, 147°30' E) (Australian Bureau of Meteorology Station) during early spring and summer of 2005-06 and 2006-07 relative to the long term average based on 1958 - 2007 (www.bom.gov.au).

Leaf emergence as a function of calendar day

The rate of leaf emergence (per day) was variable between and within seasons at all sites (Figure 3.5 – Figure 3.8). In 2006-07, there was a general decline in the rate of leaf emergence in the early part of the season until early to mid November at all sites except for Chardonnay A.

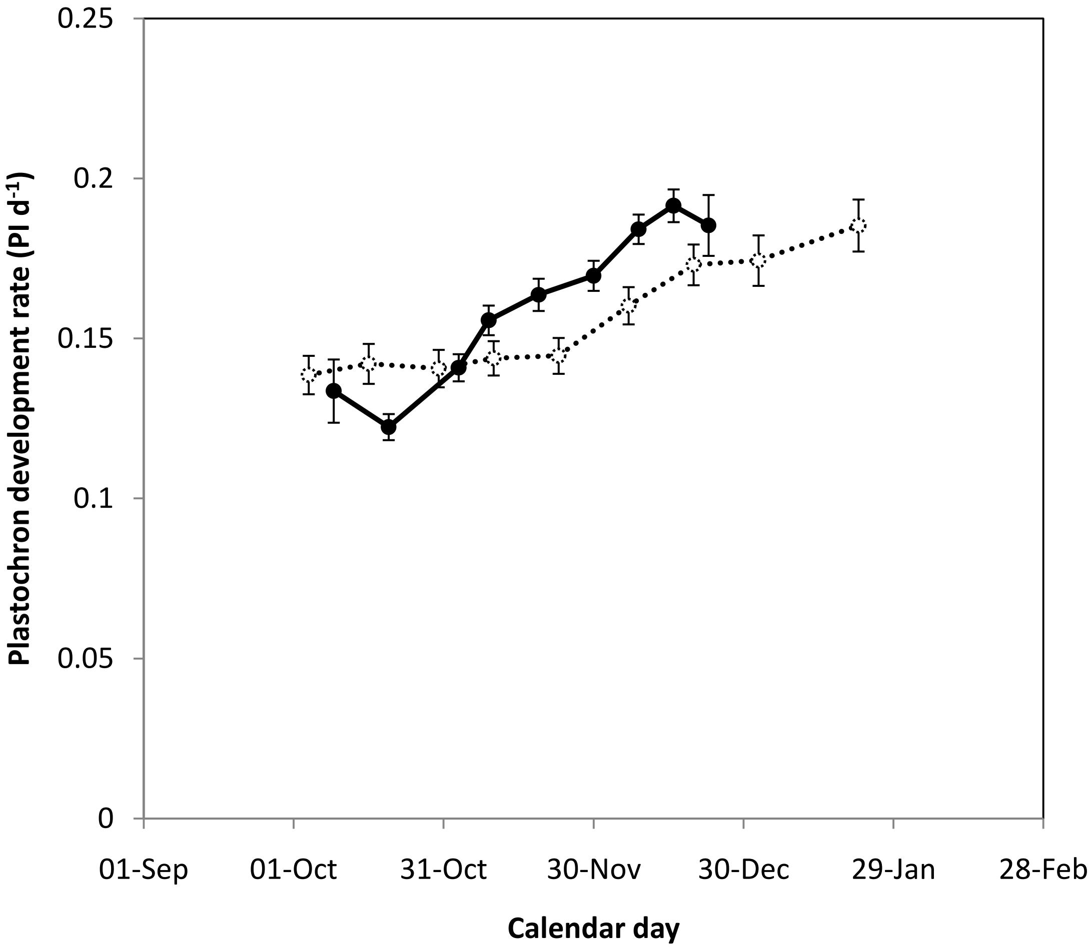

Figure 3.5. Plastochron development rate per day, from 1st September (budburst in 2005) for 2005-06 (–), 2006-07 (···) for Chardonnay A. Budburst was one week later in 2006.

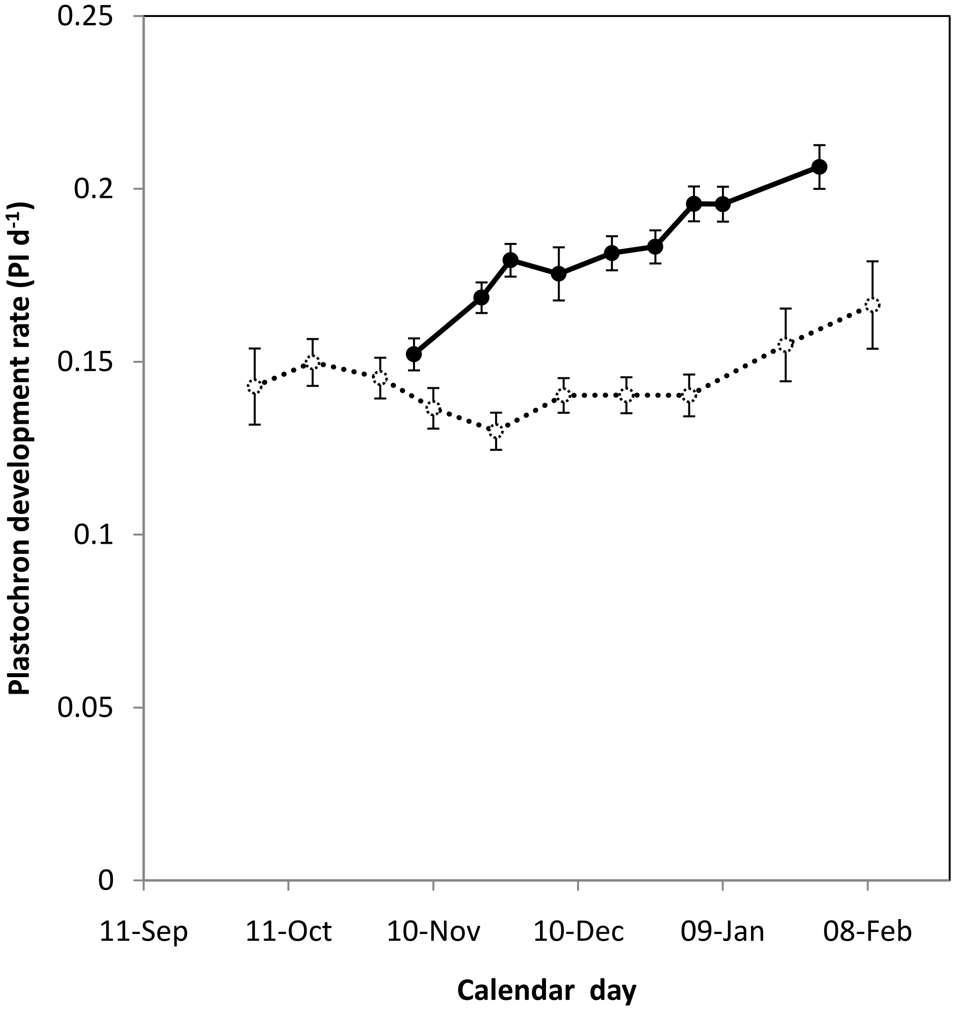

Figure 3.6. Plastochron development rate per day, from 11th September (budburst in 2006) for 2005-06 (–), 2006-07 (···) for Chardonnay B. Budburst was over 2 weeks later in 2005.

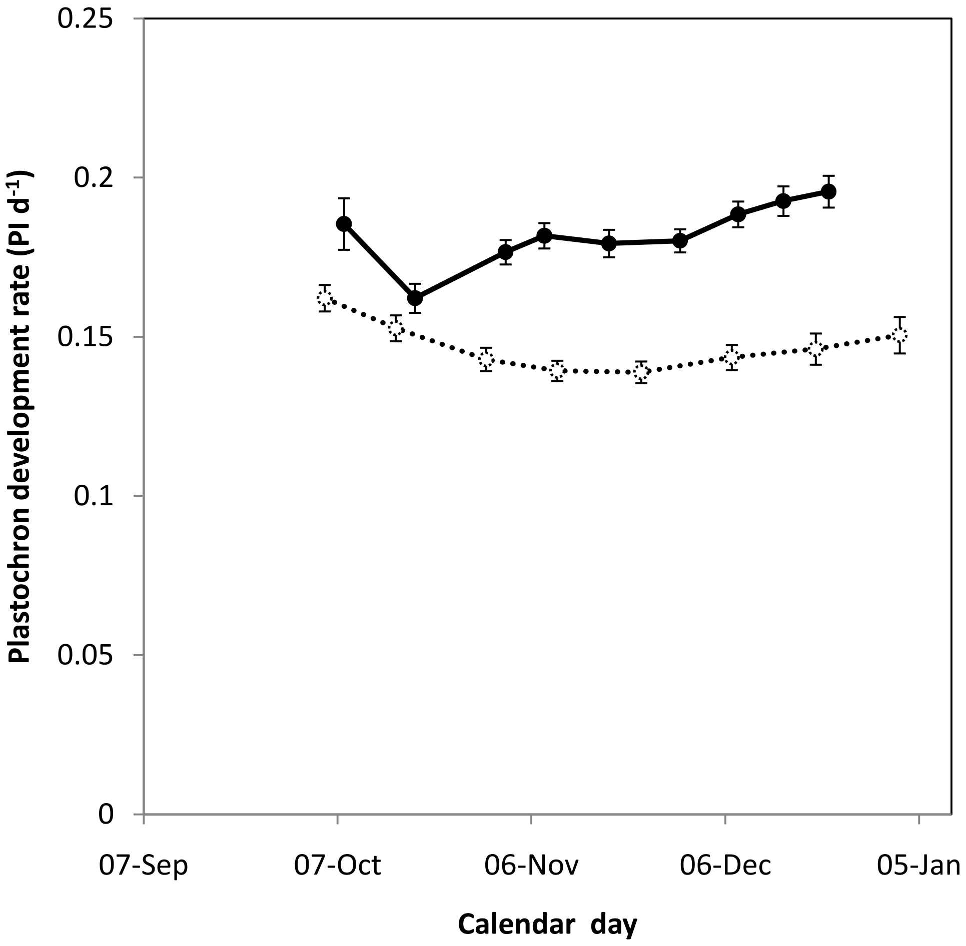

Figure 3.7. Plastochron development rate per day, from 7th September (budburst in 2006) for 2005-06 (–), 2006-07 (···) for Pinot noir A. Budburst was 5 days later in 2005.

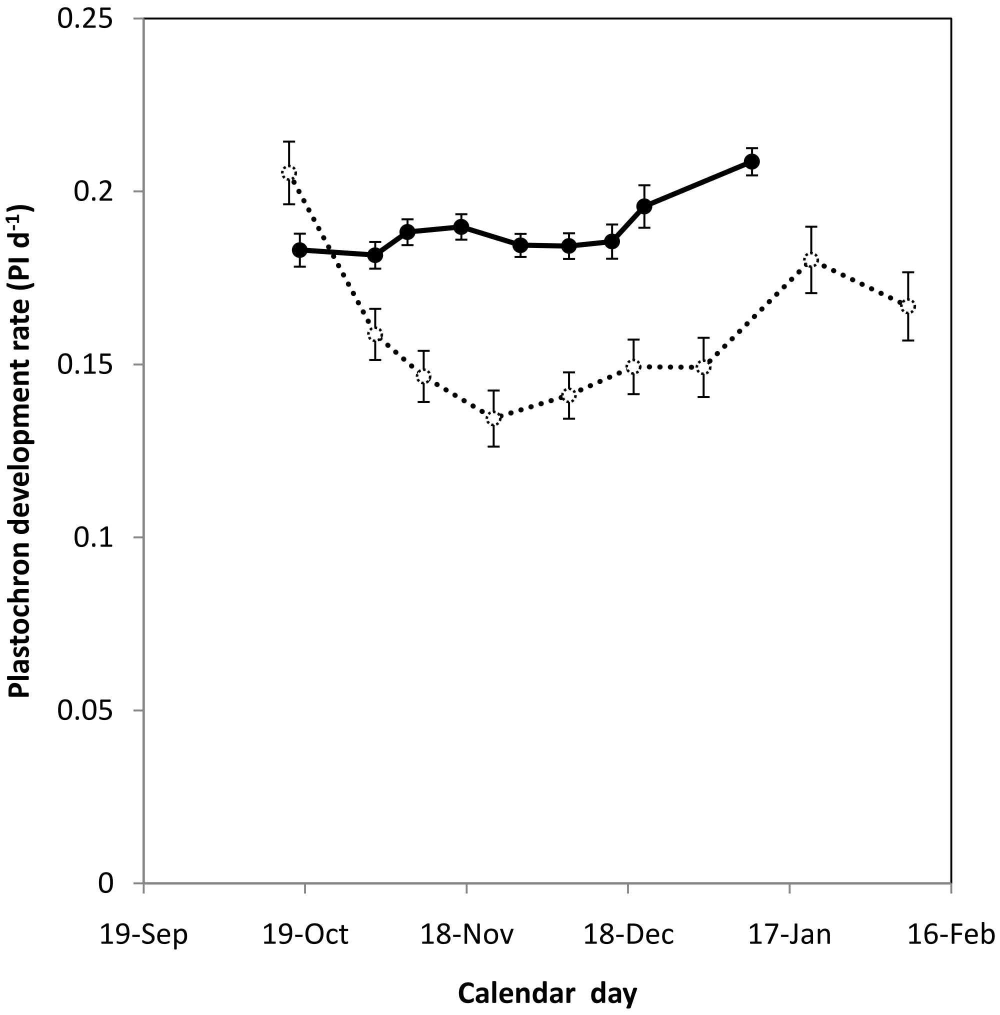

Figure 3.8. Plastochron development rate per day, from 19th September (budbust in 2005 and 2006) for 2005-06 (–), 2006-07 (···) for Pinot noir B.

Leaf emergence as a function of thermal time

Cumulative thermal time accounted for a high proportion of the variance in plastochron index according to linear regressions of all shoots at each vineyard site (refer to Appendix 2 for R^2 values). There were no significant interactions ($P > 0.05$) between any factors used in the ANOVA for the rate of leaf emergence for any site and there was no significant effect of growing season (year) on rate of leaf emergence at any site ($P > 0.05$) when it was calculated using thermal time. There was, however, a significant effect of shoot position ($P < 0.05$) on the rate of leaf appearance at both sites for Chardonnay and one of the two sites for Pinot noir (Table 3.3). At sites where there was a significant effect, medial shoots had a slower rate of leaf emergence than shoots from either distal or basal buds ($P < 0.05$). There was no significant effect ($P > 0.05$) of shoot orientation on the rate of leaf emergence at either Pinot noir site.

Table 3.3. The effect of shoot position on the rate of leaf emergence (mean plastochron index per degree day) for Chardonnay and Pinot noir vines. Figures presented are the mean of two growing seasons.

	Mean plastochron index per degree day at each block			
Shoot position	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
Distal	0.049	0.039	0.038	0.037
Medial	0.038	0.034	0.037	0.030
Basal	0.050	0.043	0.038	0.034
P value	*<0.001*	*<0.001*	*0.69*	*<0.001*
$LSD_{0.05}$	*0.004*	*0.004*	*ns*	*0.003*

Shoot growth as a function of thermal time

Cumulative thermal time accounted for a high proportion of the variance in shoot length according to linear regressions of all shoots at each vineyard site (refer to Appendix 2 for R^2 values). There were no significant interactions (*P* > 0.05) between any factors used in the ANOVA for the rate of shoot growth for any site. There was a higher rate (*P* < 0.05) of shoot growth in 2005-06 than in 2006-07 (Table 3.4). Shoot position on the arm also had a significant (*P* < 0.05) effect on the rate of shoot growth at all sites, with medial shoots having a significantly slower rate of shoot growth than distal or basal shoots (*P* < 0.05, Table 3.5).

Table 3.4. The effect of growing season on the rate of shoot growth (mean shoot length (cm) per degree day) for Chardonnay and Pinot noir vines. Figures presented are the mean of all shoot positions and orientations.

	Mean length of shoot (cm) per degree day for each block			
Season	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
2005-06	3.64	2.26	3.69	2.41
2006-07	2.42	1.40	3.01	1.41
P value	*<0.001*	*<0.001*	*0.002*	*0.002*

Table 3.5. The effect of shoot position on the rate of shoot growth (mean shoot length (cm) per degree day) for Chardonnay and Pinot noir vines. Figures presented are the mean of two growing seasons.

	Mean length of shoot (cm) per degree day for each block			
Shoot position	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
Distal	3.57	2.10	3.75	2.32
Medial	1.95	1.26	2.87	1.33
Basal	3.60	2.14	3.42	2.08
P value	*<0.001*	*<0.001*	*0.004*	*0.004*
$LSD_{0.05}$	*0.47*	*0.39*	*0.50*	*0.52*

Leaf area development as a function of thermal time

Cumulative thermal time accounted for a high proportion of the variance in leaf area according to linear regressions of all shoots at each vineyard site (refer to Appendix 2 for R^2 values). There were no significant interactions ($P > 0.05$) between any factors used in the ANOVA for leaf area development at any site. There was a significantly ($P < 0.05$) higher rate of leaf area development in 2005-06 than in 2006-07 (Table 3.6) and a significant effect of shoot position ($P < 0.05$) on the rate of leaf area development at both sites for Chardonnay and one of the two sites for Pinot noir (Table 3.7). At sites where there was a significant effect, medial shoots had a slower rate of leaf area development than either distal or basal shoots.

Table 3.6. The effect of growing season on the rate of leaf area development (mean leaf area (cm^2) per degree day) for Chardonnay and Pinot noir vines. Figures presented are means for all shoot positions and orientations.

	Mean leaf area (cm^2) per degree day for each block			
Season	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
2005-06	5.62	3.80	4.81	2.41
2006-07	3.81	2.18	4.05	1.41
P value	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Table 3.7. The effect of shoot position on the rate of leaf area development (mean leaf area (cm^2) per degree day) for Chardonnay and Pinot noir vines. Figures presented are the mean of two growing seasons.

	Mean leaf area (cm^2) per degree day for each block			
Shoot position	Chardonnay A	Chardonnay B	Pinot noir A	Pinot noir B
Distal	5.70	3.29	4.50	2.32
Medial	3.00	2.22	4.24	1.33
Basal	5.46	3.46	4.55	2.08
P value	*<0.001*	*<0.001*	*0.34*	*0.001*
$LSD_{0.05}$	*0.816*	*0.626*	*ns*	*0.524*

DISCUSSION

Budbreak occurred on the same calendar day at one site, earlier at one site and later in 2005 than 2006 at the two remaining sites and, though not measured precisely, bud burst was noted first on distal nodes, then along nodes closer to the trunk. Variability in shoot development is partly due to differences in the timing of budbreak on different nodes

(Kliewer *et al*. 1989), which results in differences in their developmental stages. The more developed shoots take advantage of their higher sink strength by absorbing more nutrients (Williams, 1987; Miller *et al*., 1996), which is thought to result in greater growth and vigour. The earlier time of budbreak of distal nodes did not translate into shoots with greater developmental or growth rates at any site or season when shoots arising from distal and basal nodes were compared. Medial shoots however had a slower rate of shoot growth at all sites and slower rates leaf emergence and leaf area except at Pinot noir A. Even at the site where there were arched canes (Chardonnay B), which is thought to reduce bud failure and improve growth of medial shoots (Rosner and Cook 1983), medial nodes produced shoots which had lower growth and development rates. This result is also in agreement with May *et al*. (1978) who found that canes arched upwards, like Chardonnay B, did not produce any benefit in terms of more uniform growth.

Grapevine shoots exhibit an indeterminate growth which can continue indefinitely unless arrested by unfavourable conditions, such as adverse temperature (Buttrose 1968), daylength (Kim and Ko 1986), water deficit or nutrient stress (Winkler 1970). The results of this study indicated that air temperature accounted for a high proportion of the variance in the rate of leaf emergence (Table 3.4). Similar results have been obtained for both glasshouse and field grown grapevines (Lebon *et al*. 2004). Conversely, Schultz (1992) showed that the rate of unfolded leaf production of the primary shoot decreased gradually during shoot development in the vineyard. Unlike the study of Schultz (1992), measurements in the current study stopped once shoots were hedged and hence a decline in the rate of leaf emergence may not have occurred before measurements ceased. Cultural practices, such as crop load, mineral nutrient (particularly nitrogen) status and water supply, that may influence growth rate (Smart and Coombe 1983) or high crop loads (Hardie and Martin 2000) were not controlled or monitored in the current study.

The plastochron development rate per calendar day varied markedly between and within seasons, confirming concerns that calendar-based spray schedules may be less effective in situations where development of the plant canopy is temporally variable. For instance, at the site Pinot noir B (Figure 3.8), where budburst was on the same calendar day in both years, from mid September until mid December in 2005-06 the plastochron development rate was in the order of 0.18 leaves developing per a day. Similarly in 2006-07, growth from mid September to mid October was over 0.2 leaves developing per day. This would translate into 5.4 leaves developing in the first month in the first season and at least 6 in the second season. However after mid October plastochron development rate was much slower in 2006-07 than 2005-07, with approximately 0.14 leaves and 0.18 leaves developing per day respectively; this translates to approximately 20% fewer leaves emerging per a day in 2006-07. This variation is likely to result in heterogeneity in the amount of untreated leaves, emerging since the last fungicide application, that are highly susceptible to infection by *E. necator*.

The development of shoots measured as leaf emergence, shoot length or leaf area development, was analysed with the response variable expressed as linear functions of thermal time, to account for temperature variability within and between seasons. This approach provided an effective way of estimating and thus predicting shoot development in response to temperature. The rate of leaf emergence, expressed as a function of thermal time, was constant from budburst to hedging (refer to Appendix 2) and between seasons at each site according to ANOVA. It was the only measure of shoot development, which did not vary with season. There was both a higher rate of shoot growth and leaf area development as functions of thermal time in the wetter season of 2005-06 than 2006-07. Rate of shoot growth and leaf area development per unit thermal time followed similar trends which was not surprising as shoot length and leaf area have been shown to be closely related (Miller *et al*. 1996; Miller and Howell 1998; Siegfried *et al*. 2007). As there was no difference in the per thermal unit rate of leaf emergence between seasons, the greater per thermal unit rate of leaf area development in 2005-06 compared with 2006-07 is attributed to larger leaves rather than a greater number leaves. This variability between seasons in the rate of leaf area produced means leaf area *per se* is not able to be predicted using thermal time in Tasmanian commercial vineyard conditions. The results suggest other factors, possibly nutrient or water status, may limit leaf expansion. The greater leaf growth in the wetter 2005-06 season confirms water status as a likely limiting factor. As noted in the thesis introduction, nutrient and water stress in commercial vineyards appears to be common. The analysis of leaf appearance rates on primary shoots calculated against thermal time, appears independent of external factors, other than temperature and may be a useful first step towards timing sprays against cumulative thermal time, rather than calendar-based spray scheduling.

Leaf production and leaf area development on the branches (lateral shoots) was not modelled. Secondary shoot development would need to be taken into consideration with a spray recommendation program as the leaves on the branches account for 10-50 % of the plant leaf area, depending on the training system, plant vigour, pruning intensity and genotype (Williams, 1987; Mabrouk *et al*., 1997; Palliotti *et al*. 2000). The effects of these multiple factors on shoot development result in considerable variability in plant architecture in grapevine (Lebon *et al*. 2004), making secondary shoot development hard to predict.

Shoot development is variable in Tasmania's climate; however, it was possible to describe shoot development in terms of rate of leaf emergence in response to temperature conditions both within and between seasons. From this research it would be possible to simulate the number of leaves on the primary shoots of the vineyards assessed in future years according to thermal temperature, a first step towards making recommendations about spray interval based on shoot development. However, secondary shoot development must be taken into account, together with primary shoot development, when developing shoot growth models. In contrast to leaf emergence, shoot length and leaf area development varied between the seasons, probably due to soil water availability under these commercial management conditions.

GENERAL DISCUSSION

In the cool climate of Tasmania, commercial grape growers experience great difficulty controlling powdery mildew in some seasons, whilst in others, the disease presents few problems. It is common for grape growers to apply the same calendar-based spray program each year regardless of the disease risk, with a typical spray interval of two weeks between successive fungicide applications. Occasionally these spray programs are sub-optimal because spray intervals were too long or not timed in relation to disease development. Against this background, the present study was based on the premise that disease development is closely related to shoot development. Results presented in Chapter 1 demonstrated that the severity of powdery mildew on grapevine foliage following a single infection event depended on the environment under which the shoot developed and provided new information about the development of ontogenic resistance to *E. necator* infection. In Chapter 3, grapevine shoot development was described for two consecutive growing seasons and was found to vary widely between seasons and also within each season, and between varieties and vineyards. Throughout the region, growers reported higher than usual disease severities in the first season of the shoot growth study and lower disease severities in the second season (Lubiana 2006; Lubiana 2007). While this difference might have been due to primary inoculum arriving earlier in the growing season in the first season, growers attributed the difference to the larger canopies in the first season resulting in an environment more conducive to disease. Issues around spray penetration and coverage were also mentioned. Based on the results of Chapter 1, the greater rate of shoot development due to greater heat summation in the first season may have also made individual leaves more susceptible to infection as well as promoting a greater area of non-protected leaves that emerged before a subsequent fungicide application. Large differences in foliage development between growing seasons appear to be a feature of the Tasmanian climate which is not accounted for by calendar-based spraying.

Inoculation of all leaves of a Cabernet sauvignon shoot with *E. necator* conidia under conditions favourable for both pathogen and host growth (average of 25^{o}C in a humid glasshouse) revealed how disease severity initially increased and then decreased with increasing leaf position (Chapter 1). Examination of early fungal development revealed the leaf positions for which the frequency of primary germ tube formation and the development of secondary hyphae was being reduced, with the latter showing a similar response to disease severity as a function of leaf position. Ontogenic resistance was demonstrated because there was no visible disease or leaves with secondary hyphae beyond leaf position 18. The formation of secondary hyphae, even for a low percentage of conidia observed, indicated cuticle penetration and some level of colony development.

A mechanistic model, developed using Bayesian inference, provided a quantitative description of ontogenic resistance and a means of separating the effects of vine leaf resistance and pathogen growth (component models). Significant differences in the parameter estimates of the pathogen growth model for the two pre-inoculation environments tended to support the hypothesis that the pre-inoculation environment had a direct effect on some determinant of the 'quality' of plant tissue to be colonised by *E. necator*, noting that shoots which were growing rapidly before infection were more susceptible to pathogen attack than leaves on shoots which developed more slowly. Agrios (2005) suggested that when the optimum temperature for both pathogen and host are similar, then the host plant will not be able to grow in advance of pathogen colonisation and escape disease. Conversely, when the host plant is growing slowly at temperatures that are sub-optimum, then the pathogen will also develop slowly. The results of Chapter 1 suggest a more complex situation than outlined in Agrios' (1995) introductory text on plant pathology. In terms of an epidemic, rapid shoot growth might have a twofold effect on a secondary cycle of infection; that is, not only will a greater proportion of host tissue be susceptible to infection, but the resident pathogen may also be growing rapidly and sporulating abundantly at optimum temperatures and relative humidities. The latent period for *E. necator* at 25-30^{o}C can be as short as 5-6 days in controlled environments (Delp 1954) and 7-10 days at 25^{o}C in the field (Gubler *et al*. 1999), with an ideal relative humidity for disease incidence and severity of 85% (Carroll and Wilcox 2003). Thus, leaves which have grown under conditions making them highly susceptible to infection might be exposed to a higher inoculum load than plant canopies with slower rates of leaf emergence, especially if sporulation is promoted by high relative humidity (Aust and Hoyningen-Huene 1986) in a dense vine canopy.

Experiments conducted in glasshouses tend to overestimate the disease susceptibility of shoots, which could make it difficult to relate the results found in Chapter 1 to field conditions. This could be due to interactions between environmental and host factors during shoot development and the method of inoculation. For instance, Moyer *et al*. (2010) found that *V. vinifera* leaf tissue exposed to cold temperatures (≤ 8ºC for 2 to 8 h) reduced infection efficiency and colony expansion when tissues were subsequently inoculated. In the field, single spores are deposited on the leaf surface and not at the high densities that are generally provided by inoculation with conidial suspensions or dry conidia from detached leaf cultures. Gadoury *et al*. (2010) found that conidial density affected the latent period of *E. necator*, which decreased exponentially as the number of germinable conidia increased from 1 per mm^2 until approximately 10 to 20 germinable conidia per mm^2, with higher densities causing a slight decline in latent period. Additionally, plants in glasshouse studies are not constantly exposed to inocula because shoots are inoculated when there is a sufficient area of highly susceptible leaves. It would be interesting to repeat experiments conducted in this study with shoots of different development stages to see the response of plant development on the expression of leaf ontogenic resistance.

The cause of leaf ontogenic resistance and the reason why conidial germination with secondary hyphae on Cabernet sauvignon leaves ceased at leaf positions > 17 remains obscure. As such, the research focused on the question of what makes a leaf highly susceptible to infection by *E. necator*. A more detailed study of leaf 'quality' during growth showed that Cabernet sauvignon leaves were most prone to development of severe powdery mildew infection immediately after they had ceased to import carbohydrate (Chapter 2). This relationship was maintained when plants were subjected to different pre-treatment environments. Low disease severities on newly unfolded leaves that were 'sinks' for carbohydrate might have been the result of a number of factors. Rapid expansion of leaf area, and hence 'dilution' of pathogen biomass, was unlikely to be the only cause of reduced disease severities because the modal leaf position for the maximum percentage of conidia with secondary hyphae was > 4. It seems likely that the activation of secondary biochemical pathways required for induced defence was constrained in very immature leaves that utilise most of their own energy for growth (Herms and Mattson 1992) as it has been shown that the induction of defence is cost-intensive in several pathosystems (Heil and Bostock 2002; Swarbrick *et al.* 2006; Todesco *et al*. 2010).

Clearly, an important question is whether carbohydrate status alone is responsible for variation in powdery mildew susceptibility on leaves as they develop. The timing of this transition varies with both crop and cultivar but is related to leaf age (Yang and Hori 1980) and marks a fundamental transition in the physiology of the leaf (Turgeon 1989). During the early stages of leaf growth there is a high respiratory need as primary metabolism requires synthesis of relatively high amounts chlorophyll, proteins and structural compounds. As the photosynthetic system matures the catabolic rate slows (Marchi *et al*. 2005). Results from Iacono and Sommer (2000) suggest grapevine leaves experience a tri-phasic pattern of photosynthesis in relation to leaf age. There is first a rapid increase to a maximum rate, which then plateaus before being followed by a non-linear decline in the photosynthetic rate. The powdery mildew fungi, co-evolved intimately with their host plants, appear to have found a mechanism to acquire readily available photosynthate for obligate parasitism.

The results obtained here present a strong argument for utilization by the fungus of available carbohydrate at the sink/source transition, with the biotrophic organism becoming a nutrient sink by directing the flow of carbon within the host plant to the infection site (Brem *et al*. 1986). The interaction between the host and pathogen results in many changes in physiological processes (Moriondo *et al*. 2005) beyond the scope of the present study. Brem *et al*. (1986) observed increased sugar concentrations in grapevine leaves infected by *E. necator* and *P. viticola* but carbon fixation was decreased in infected tissue. Caffi *et al*. (2010) found a similar reduction in net assimilation rate for leaves infected with *P. viticola* and this occurred before visible symptoms of disease. Brem *et al*. (1986) also observed an enhanced invertase activity, the origin of which was not established in the study reported, and postulated that it was the mechanism by which the oomycete acquired carbon, thus altering the natural physiological relationships between source/sink leaves in the plant. Hibberd *et al*. (1996) also found that photosynthesis is reduced after the accumulation of invertase.

In order to obtain a more detailed understanding of the physiological relationships between the perennial host and biotrophic pathogen, it is important to consider the vine growth sequence through vegetative shoot extension and development of reproductive organs (flowers and fruit). For interdeterminate plants such as grapevine, where there is no clear delineation between vegetative and reproductive growth, there is competition for assimilates between vegetative and reproductive organs. Petrie *et al*. (2000) suggested that, after veraison, the demand for carbohydrates may be greater than the leaf area established can provide, resulting in the vine being considered source-limited. In the present study, which used one year-old plants, there was no developing fruit to compete for assimilates and further work is needed to clarify relationships between crop load or stage of crop development and susceptibility of leaves to infection. A hypothesis that could be tested is that young leaves formed later in the season are less susceptible to infection by *E. necator* than leaves formed before anthesis and fruit development, when there was less intense competition for assimilates. The marked difference in the final area of individual leaves between the two seasons of the field trials and the lack of any effect of different glasshouse conditions (in the two trials) on the same measure also points to a need to carefully assess the effects of irrigation management in particular. The observation, from the Introduction to the thesis, that commercial growers tend to run vineyards at suboptimal irrigation and fertilizer regimes to enhance wine quality, may lead to enhanced impacts of seasonal conditions on the variability of leaf growth and hence susceptibility.

Further research on the relationship between *E. necator* and the sink-source transition should also consider studies at a 'within lamina' scale. During the most rapid phase of lamina expansion, there is a simultaneous import and export of photosynthates (Larson and Dickson 1973), and, physiologically, the leaf is not homogenous. Moreover, carbon assimilation may be segregated in different regions of the lamina during development (Larson and Dickson 1973). For instance a mature tip of a leaf can be physiologically analogous to a mature leaf (Larson and Dickson 1973; Turgeon 1989) whilst the remaining area may continue to act as a typical immature leaf sink. Future studies investigating mechanisms of ontogenic resistance in relation to the photosynthetic sink to source transition will require greater spatial and temporal resolution of these processes in leaves.

If carbohydrate status is an important factor in the susceptibility of leaves to infection, many cultural practices will also affect leaf susceptibility. Vine manipulation designed to adjust grape quality may either inadvertently or intentionally alter source-sink relationships. Measures designed to increase bunch exposure to light and thus improve grape skin characters that contribute to wine colour, including leaf plucking, shoot tipping and tucking canes, all reduce actual or photosynthetically effective leaf area. Similarly, increasing the relative size of the reproductive versus the vegetative sink by bunch thinning may, depending on timing, substantially alter the reproductive sink strength reducing demand for leaf photosynthate, which in turn might allow the shoot to put more energy into growth so that leaves become relatively more susceptible to infection. Alternatively, the additional carbohydrate supply may be utilised by the host to energise defence or to incite repair mechanisms to better cope with infection or wounding (Truernit *et al* 1996). The regulation of sucrose and starch formation is also affected by environmental conditions and by changes in source-sink relations within the vine (Mullins *et al*. 1992). For example, leaf temperature affects the relative concentrations of sucrose and starch within the leaves of grapevine (Buttrose and Hale 1971).

Asynchronous shoot development (Chapter 3) in Chardonnay and Pinot noir was partly the consequence of medial shoots having a slower rate of leaf emergence than shoots that were proximal or distal to the vine trunk. From the present results, this asynchrony is likely to be related to disease development and severity in the field because heterogeneity in leaf development would cause variation in the timing of when leaves acquired ontogenic resistance. Such variation does not appear to have been considered in previous studies (e.g. Calonnec *et al*. 2008) and could be accounted for in the development of spray programs that use shoot growth models. This study also revealed that the relationship between plastochron index and thermal time can be developed for a particular site by calculating the average slope regression coefficient from the rate of leaf emergence for multiple, individual shoots. As the rate of leaf emergence for a particular shoot position did not differ between seasons at each experimental site studied, measurements taken in one growing season only should be sufficient to develop a site-specific prediction for the rate of leaf emergence per unit of thermal time. Prediction of the highest rates of leaf emergence among shoots within a vineyard site should be used for developing the decision rule for timing fungicide applications to ensure that none of the (fastest growing) shoots at a site develop unacceptably large areas of unprotected, susceptible leaf tissue. Based on this research, leaf emergence rates predicted from the mean of the basal and distal shoots at each site should be selected for this purpose. This study revealed that the relationship between plastochron index and thermal time can be developed for a particular site by calculating the average slope regression coefficient from the rate of leaf emergence for multiple, individual shoots. As the rate of leaf emergence for a particular shoot position did not differ between seasons at each experimental site studied, measurements taken in one growing season only should be sufficient to develop a site-specific prediction for the rate of leaf emergence per unit of thermal time.] The next step in this research would be to determine how many new leaves can emerge on distal and basal shoots without needing fungicide protection while still maintaining an economically desirable level of disease control. Bleyer (2001), for example, found that the number of fungicide applications could be reduced significantly without affecting downy mildew control by using the decision rule to spray after four new leaves had been predicted to develop following the last fungicide treatment. This approach would need to be tested empirically by comparing the level of disease control achieved using sprays applied according to a derived decision rule versus a standard calendar-based spray program.

Using knowledge generated in Chapter 1, it should also be possible to estimate the proportion of leaves on a grapevine shoot that can potentially develop macroscopic signs of powdery mildew after first-time exposure of a shoot to *E. necator* conidia. Assuming the leaf with a lamina length of approximately 90% of its final length is the 'maximum severity' leaf (Chapter 2), then the symmetrical shape of the responses detailed in Figure 1.3 suggests that counting the number of leaves younger than the 'maximum severity' leaf but with a lamina length of ≥ 30 mm will provide a figure which then equals the number of leaves older than the 'maximum severity' leaf that would be expected to show visible signs of powdery mildew infection. This approach might be problematic for shoots with only 3-4 expanded leaves that are yet to reach full size (data not presented). Nevertheless, this type of information could then be used in simulation models, such as the PLS-path modelling described by Calonnec *et al.* (2010), to ascertain the effect of the time of the first leaf infection and viticultural factors that influence the number and position of leaves infected in relation to the time of flowering and the severity of powdery mildew on bunches. Results from Chapter 1 and those of Moyer *et al.* (2010) highlight that more research is required to predict the relative severity of powdery mildew on leaves in relation to the environment in which shoots develop.

The liberal use of fungicides has resulted in some chemicals no longer offering good control in some grape-growing regions (Erickson and Wilcox 1997). Available fungicides need to be applied in a more strategic manner to prolong the period (number of years) in which they remain effective. Quantification and understanding of leaf ontogenic resistance in grapevine is important for explaining observed differences in disease development in the field. With greater knowledge, spraying programs might be tailored to account for variation in vine phenology and shoot and fruit development associated with changes in disease susceptibility. Control measures may be aligned better with the actual risk of infection and potential loss (Ficke *et al*. 2002). Ontogenic resistance of both leaves and fruit offers a means of targeting spray applications and reducing spray intervals when the canopy is highly susceptible to infection during periods of rapid canopy development. As noted in the General Discussion, models of ontogenic resistance will have to be adjusted for site and variety, taking into consideration asynchronous shoot development associated with particular pruning and training systems. The final section of this thesis summarises recommendations for future research.

Recommendations for Future Research

1. Quantify leaf ontogenic resistance again but with plants at different developmental stages of vegetative and reproductive development; for example, 6 leaves, 12 leaves and 20 leaves, with and without fruit at various developmental stages; for example, anthesis, fruit set and veraison.
2. Quantify leaf attributes such as cuticle thickness and cell wall lignifications as leaves mature, as a function of temperature and other potentially variable management factors at which leaves develop, to ascertain what characters, as influenced by environmental conditions, are correlated to ontogenic resistance.
3. Quantify ontogenic resistance in other varieties of *V. vinifera*. Are there differences among grapevine varieties in the expression of ontogenic resistance?
4. Validate the cultivar and site-specific models for predicting the rate of leaf emergence on primary grapevine shoots by collecting data in additional growing seasons from the same sites where the models were developed to compare observed and predicted estimates of the rate of leaf emergence.
5. Test the breadth of application of the cultivar and site-specific leaf emergence models by comparing observed and predicted estimates of the rate of leaf emergence using data from the same grapevine varieties growing on the same trellis system but at different sites in the same viticultural region.
6. Derive a decision rule from validated leaf emergence models for determining the thermal time between fungicide applications for adequate leaf protection. Test various intervals of thermal time in fungicide timing trials to identify the interval that allows commercially acceptable control of powdery mildew on leaves relative to a non-treated control treatment and a calendar-based fungicide program. A spray program for protecting the inflorescence from *E. necator* infection, including short spray intervals from pre-flowering to early fruit set, should be superimposed on all treatments during field testing.

7. Conduct fundamental research to explain why grapevine leaves are most susceptible to infection by *E. necator* when they are emerging from the carbohydrate sink-to-source transition. This research may reveal novel mechanisms for preventing infection of grape leaves and lead to new disease management options for many biotrophic plant pathogens infecting woody, perennial plants.

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Predicting leaf length in *Vitis vinifera L.* A non-linear mixed-effects model.

Greg Lee

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1 Aim

Interest lies in determining the predicted final leaf area for leaves which have been destructively sampled. As a well-known relationship between leaf area and leaf length exists (Schultz, 1992), modelling focuses on the prediction of lamina leaf lengths.

2 Introduction

Leaves on grapevines emerge systematically, taking alternate sides of a growing vertical shoot. In the scenario where vines have been pruned to retain a single shoot the leaf position index $j = 1, 2, ..., J_i$ indicates the order of emergence and J_i is the total number of leaves observed on the i-th vine, $i = 1, 2, \ldots, I$.

We adopted a non-linear mixed-effects modelling approach based on a logistic growth curve function (2), and implemented this using the `nlme` package (Pinheiro et al., 2009) in the R statistical environment (R Development Core Team, 2010).

2.1 Complete pooling

As it is considerably easier to appreciate the basic form of the model in the absence of mixed-effects notation, we begin by presenting the model in a form which ignores variability between vines and leaves within vines, such as one might expect in ordinary non-linear regression (Ratkowsky, 1983; Seber and Wild, 2003)

$$y_{ijk} = \mu + \varepsilon_{ijk} \tag{1}$$

where the response y_{ijk} is a vector of $k = 1, 2, \ldots, K_{ij}$ repeated measures recording the length of the j-th leaf from the i-th vine, μ is the model mean (2), and ε_{ijk} are the residual deviations of individual data points from the model mean. Implicit in (1) is the assumption that the residuals arise independently from a normal distribution with mean zero and constant variance, which we write as $\boldsymbol{\varepsilon} \sim \mathcal{N}(0, \sigma^2)$. The mean response μ is a symmetrical sigmoidal curve determined from the three parameter logistic function

$$\mu = f(x_k, \boldsymbol{\beta}) = \frac{\phi_1}{1 + \exp[(\phi_2 - x_k)/\phi_3]}, \tag{2}$$

where the x_k are the series of repeated leaf length measurements taken at discrete times $k = 1, 2, \ldots, K$. The model parameters $\boldsymbol{\phi} = (\phi_1, \phi_2, \phi_3)$ can be interpreted as follows

- ϕ_1 is the horizontal asymptote as $k \rightarrow \infty$,
- ϕ_2 is the inflection point of the curve, the value of t at which the response attains $\phi_1/2$
- ϕ_3 is a scale parameter representing the distance on the abscissa between ϕ_2 and the point where the response attains $\phi_1/(1 + e^{-1}) \sim 0.73\phi_1$.

Primary interest lies in obtaining values of ϕ_1 for specific leaves which have been destructively sampled after inoculation with *E. necator*. As written in (1) and (2) the model specification suggests a single value for each of the parameters. This is equivalent to suggesting that all leaves will have the same predicted final length across all vines, and does not satisfy the primary goal of the research. We therefore need to extend the model to allow predictions for individual leaves.

2.2 No Pooling

One way that we might do this is to develop a model in which each individual leaf is used to estimate the model parameters separately. Under this scheme the parameters of (2) might appear as $\boldsymbol{\phi} = (\phi_{1ij}, \phi_{2ij}, \phi_{3ij})$ to indicate that individual estimates are available for the j-th leaf from the i-th vine. There is no sharing of information between leaves, as each leaf determines the parameter estimates in isolation from the remainder. Note that the number of parameters required to summarize the model grows linearly with the sample size $p = 3n + 1$, making a concise summary of the model increasingly difficult as the data grow large.

2.3 Mixed Model

Here we develop a model which represents a compromise between the complete pooling and no pooling approaches. It incorporates an average response for the population of leaves sampled and allows for individual variations from this average to be attributed at both the vine and leaf levels. A "fixed-effect" component represents the average response in the sample, analogous to the single parameter estimates implied by equations (1) and (2), and a "random-effect" component allows for the heterogeneity displayed by vines and leaves within vines. The random-effects are such that the series of data from each leaf will allow an individual estimate of it's (asymptotic) length at maturity. Because the model contains both "fixed-" and "random-" effect types, it is called a "mixed-effects" model. West et al. (2007) provide an accessible entry point into this modelling framework and provide practical guidance on implementing linear mixed-models in a range of popular software. Pinheiro and Bates (2000) provide a detailed account of the popular implementation available in the `R` statistical environment, including the non-linear extensions used here. A more theoretical treatment is offered by McCulloch et al. (2008).

2.4 Data

The data $\boldsymbol{y}$ records the length of individual leaves over time, and are grouped according to a) the position of the leaf on the shoot, b) the vine on which the shoot occurs, and c) the temperature treatment imposed on the vine. A total of 6785 observations were made on leaves from 88 vines across 2 temperature treatments.

Leaves with fewer than four distinct length measurements provide insufficient data to estimate the parameters in (2) and were excluded from the model. The final number of leaves used was 722.

Because the vines were subject to temperature treatments and these were expected to have a differential effect on vine growth, *thermal time* – a daily integration of temperature over time measured in units of degree-days (°Cd) (as described in Pallas et al., 2009, for example) – was used as the primary covariate rather than simple clock time.

Because the present study emphasises the growth of individual leaves, thermal times were adjusted to measure elapsed thermal time for each leaf. That is, each leaf has a first measure of thermal time at $t = 0$.

2.5 Modelling Strategy

The model-building strategy adopted here loosely follows that presented by Pinheiro and Bates (2000). Specifically, we opt for building many simple models rather than fewer, more elaborate models. We engage a process of model criticism for each candidate model as a means of identifying potential refinements. Where possible, likelihood ratio tests are used as the mechanism for identifying a preferred candidate, and unless there is a specific case for an alternative, otherwise equivalent models are chosen based on parsimony.

3 Model Building

To allow predictions for final asymptotic leaf lengths of individual leaves, we need to incorporate model parameters capable of varying between leaves. We re-write (1) and (2) as the mixed-effects model

$$\begin{aligned}
\boldsymbol{y}_{ijk} &= \frac{\phi_{1ij}}{1+\exp[-(t_{ijk}-\phi_{2ij})/\phi_{3ij}]} + \epsilon_{ijk} \\
\boldsymbol{\phi} &= \begin{bmatrix} \phi_{1ij} \\ \phi_{2ij} \\ \phi_{3ij} \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \begin{bmatrix} b_{1i} \\ b_{2i} \\ b_{3i} \end{bmatrix} + \begin{bmatrix} b_{1ij} \\ b_{2ij} \\ b_{3ij} \end{bmatrix} = \boldsymbol{\beta} + \boldsymbol{b}_i + \boldsymbol{b}_{ij}, \qquad (3) \\
& b_i \sim \mathcal{N}(0, \psi_{vine}), \qquad b_{ij} \sim \mathcal{N}(0, \psi_{leaf}), \qquad \epsilon_{ijk} \sim \mathcal{N}(0, \sigma^2),
\end{aligned}$$

where the model parameters ϕ_{Nij}, $N = 1, 2, 3$ have the same interpretation as provided previously, but are now allowed to vary with the grouping structure in the data – that is, separate predictions are available for individual vines and leaves. Here a nested grouping structure, namely vines $i = 1, 2, \ldots, I$, and leaves within vines $j = 1, 2, \ldots, J_i$, seems appropriate (and indeed is validated by the model fitting process). The fixed effects $\boldsymbol{\beta} = (\beta_1, \beta_2, \beta_3)$, represent the average value of the individual parameters ϕ_{Nij} in the sample of measured leaves, and the random effects represent the deviations of the vine-level b_i and leaf-level b_{ij} parameter values from the fixed effect estimates. The random effects are assumed to be independent for different vines, and leaves within vines, and the within-group errors ϵ_{ijk} are assumed to be independent for different i, j and independent of the random effects. As previously, the response data y_{ijk} represent $k = 1, 2, \ldots, K_{ij}$ repeated measures on the j-th leaf of the i-th vine.

4 Results

4.1 Fixed Effects Estimates

The fixed effects estimates $\boldsymbol{\beta}$ provide an indication of baseline parameter values for the population of measured leaves. The model fitted here does not differentiate between pre-inoculation temperature treaments. These estimates are shown in Table 1.

β_1	β_2	β_3
98.95	36.33	39.71

Table 1: Fixed-Effect Estimates

4.2 Random Effects Predictions

Random effects were included in the final model at two (nested) levels, corresponding to the parameters ϕ_{Ni} and $\phi_{Nij}, N = 1, 2, 3$, in equation (3), for the j-th leaf on the i-th vine. The variability of the random effects associated with the final leaf length parameters ϕ_{1i} and ϕ_{1ij} are of particular interest in the current application, as these indicate the level of variability one might expect in final leaf lengths at the vine and leaf levels respectively. The standard deviations associated with these random effects were determined to be 7.33 and 12.35, indicating that the majority of variation in final leaf lengths occurred at the leaf, rather than vine, level. The residual standard deviation of the model was 2.14, with the relatively small size of this variance component indicating that the majority of variability in the data was successfully captured in the model.

4.3 Predicted Final Lengths of Target Leaves

In cases where too few data were available, the final (asymptotic) lengths of target leaves were unable to be predicted by the model. Of 48 target leaves, 10 final leaf lengths were able to be predicted, and are provided in Table 2. Individual predictions at the vine ϕ_{1i} and leaf within vine ϕ_{1ij} levels have been rounded to the nearest millimetre, and this rounding accounts for the fact that some of the final predictions shown in column 6 are not exactly equal to $\beta_1 + \phi_{1i} + \phi_{1ij}$. The final column provides the maximum observed value of laminar length as a percentage of predicted final length.

Vine/Leaf	t	β_1	ϕ_{1i}	ϕ_{1ij}	Pred.	Max Obs.	% Pred.
181/11	18	99	6	-4	100	90	90.0
1810/14	18	99	2	1	102	95	93.1
1820/14	18	99	8	-11	96	84	87.5
1822/11	18	99	12	3	114	106	93.0
183/11	18	99	2	-1	100	96	96.0
1830/6	18	99	-1	13	112	100	89.3
1836/6	18	99	0	11	110	94	85.5
1838/7	18	99	8	8	116	99	85.3
188/11	18	99	6	-19	86	82	95.3
2523/13	25	99	-6	2	95	93	97.9

Table 2: Final Target Leaf Length Predictions

The predicted growth curves for the target leaves are shown in Figure 1. The excellent agreement between the predicted growth curves and the data attests the adequacy of the model.

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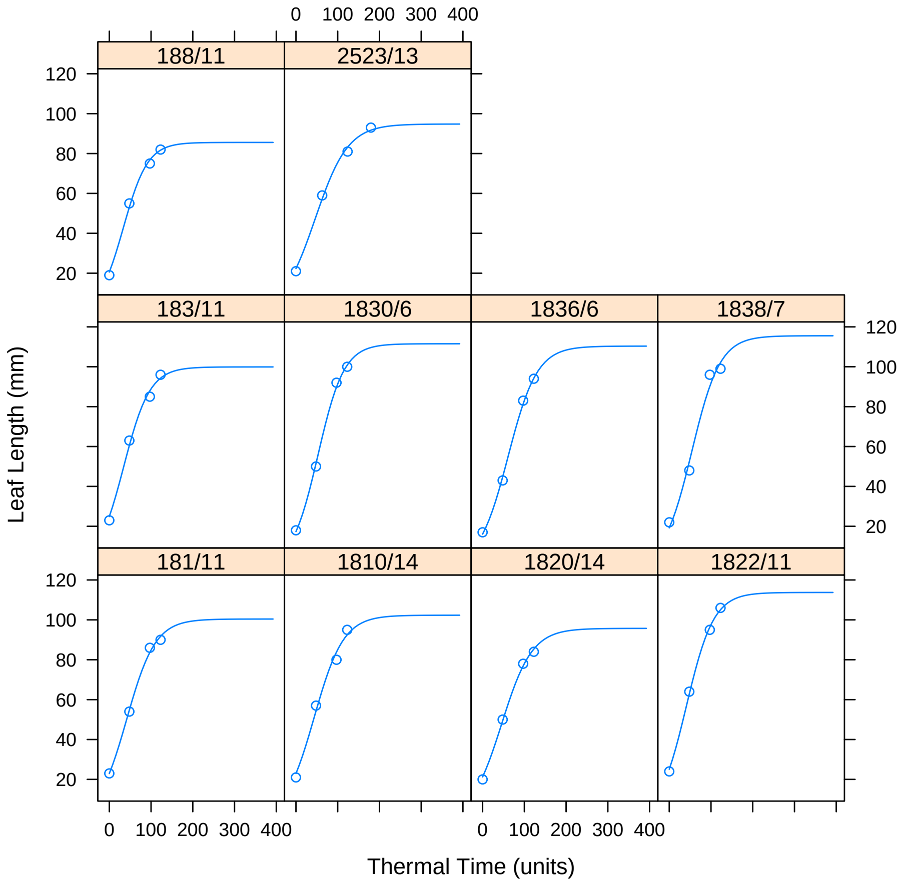

Figure 1: Predicted Growth Curves for Target Leaves

Table A1. Regression constants and coefficients of determination for plastochron index, shoot length (cm) and leaf area (cm^2) against cumulative thermal time. See main text for full details of treatments. Position refers to bud position on the overwintering arm, with basal, medial and distal buds selected. In Pinot noir, shoots on the lower arms were oriented downwards, whilst those on the upper arm were allowed to grow upwards, thus giving a horizontal split in the canopy and an additional factor in the trial design. Regressions are based on a minimum of four points (see text) and a maximum of nine in 2005-06 and ten in 2006-07.

Plastochron index						
Block	**Season**	**Shoot**	**Shoot orientation**	**Slope**	**Intercept**	**R^2**
Chardonnay A	2005-06	Distal	-	0.051	3.50	0.987
Chardonnay A	2005-06	Distal	-	0.052	3.17	0.993
Chardonnay A	2005-06	Distal	-	0.050	3.41	0.994
Chardonnay A	2005-06	Distal	-	0.049	2.48	0.981
Chardonnay A	2005-06	Distal	-	0.049	2.40	0.992
Chardonnay A	2005-06	Distal	-	0.051	3.53	0.988
Chardonnay A	2005-06	Distal	-	0.050	3.29	0.988
Chardonnay A	2005-06	Distal	-	0.037	2.63	0.987
Chardonnay A	2005-06	Distal	-	0.051	3.18	0.983
Chardonnay A	2005-06	Medial	-	0.038	1.15	0.991
Chardonnay A	2005-06	Medial	-	0.046	1.15	0.962
Chardonnay A	2005-06	Medial	-	0.045	0.64	0.997
Chardonnay A	2005-06	Medial	-	0.032	2.65	0.952
Chardonnay A	2005-06	Medial	-	0.044	2.51	0.988
Chardonnay A	2005-06	Medial	-	0.046	2.62	0.991
Chardonnay A	2005-06	Medial	-	0.053	1.94	0.991
Chardonnay A	2005-06	Medial	-	0.040	1.26	0.988
Chardonnay A	2005-06	Medial	-	0.046	3.56	0.959
Chardonnay A	2005-06	Medial	-	0.024	3.62	0.966
Chardonnay A	2005-06	Medial	-	0.042	0.40	0.964
Chardonnay A	2005-06	Medial	-	0.047	0.43	0.996
Chardonnay A	2005-06	Basal	-	0.049	3.69	0.990
Chardonnay A	2005-06	Basal	-	0.045	2.06	0.965
Chardonnay A	2005-06	Basal	-	0.048	3.12	0.989
Chardonnay A	2005-06	Basal	-	0.050	1.79	0.998
Chardonnay A	2005-06	Basal	-	0.049	1.74	0.998
Chardonnay A	2005-06	Basal	-	0.055	0.27	0.991
Chardonnay A	2005-06	Basal	-	0.052	1.97	0.996
Chardonnay A	2005-06	Basal	-	0.051	1.55	0.996
Chardonnay A	2005-06	Basal	-	0.052	3.76	0.981
Chardonnay A	2005-06	Basal	-	0.049	1.37	0.998
Chardonnay A	2005-06	Basal	-	0.050	-0.20	0.995
Chardonnay A	2006-07	Distal	-	0.050	1.89	0.984
Chardonnay A	2006-07	Distal	-	0.047	0.39	0.994
Chardonnay A	2006-07	Distal	-	0.048	1.58	0.988
Chardonnay A	2006-07	Distal	-	0.052	2.09	0.988

Chardonnay A	2006-07	Distal	-	0.046	3.27	0.990
Chardonnay A	2006-07	Distal	-	0.053	0.99	0.994
Chardonnay A	2006-07	Distal	-	0.048	1.61	0.998
Chardonnay A	2006-07	Distal	-	0.051	-1.00	0.986
Chardonnay A	2006-07	Distal	-	0.043	2.15	0.996
Chardonnay A	2006-07	Medial	-	0.048	1.93	0.971
Chardonnay A	2006-07	Medial	-	0.031	1.22	0.984
Chardonnay A	2006-07	Medial	-	0.035	1.50	0.987
Chardonnay A	2006-07	Medial	-	0.019	2.49	0.951
Chardonnay A	2006-07	Medial	-	0.047	0.75	0.995
Chardonnay A	2006-07	Medial	-	0.042	1.76	0.978
Chardonnay A	2006-07	Medial	-	0.035	0.10	0.996
Chardonnay A	2006-07	Medial	-	0.027	-0.09	0.987
Chardonnay A	2006-07	Medial	-	0.030	1.00	0.988
Chardonnay A	2006-07	Basal	-	0.061	-0.72	0.961
Chardonnay A	2006-07	Basal	-	0.051	0.37	0.994
Chardonnay A	2006-07	Basal	-	0.049	0.29	0.993
Chardonnay A	2006-07	Basal	-	0.047	1.62	0.947
Chardonnay A	2006-07	Basal	-	0.055	0.32	0.994
Chardonnay A	2006-07	Basal	-	0.046	1.53	0.991
Chardonnay A	2006-07	Basal	-	0.047	2.21	0.992
Chardonnay A	2006-07	Basal	-	0.052	1.05	0.921
Chardonnay A	2006-07	Basal	-	0.045	2.17	0.986
Chardonnay B	2005-06	Distal	-	0.039	5.00	0.991
Chardonnay B	2005-06	Distal	-	0.037	6.05	0.981
Chardonnay B	2005-06	Distal	-	0.035	5.63	0.960
Chardonnay B	2005-06	Distal	-	0.024	4.37	0.983
Chardonnay B	2005-06	Distal	-	0.047	3.68	0.998
Chardonnay B	2005-06	Distal	-	0.046	2.79	0.996
Chardonnay B	2005-06	Distal	-	0.036	4.37	0.915
Chardonnay B	2005-06	Distal	-	0.033	4.64	0.995
Chardonnay B	2005-06	Distal	-	0.040	4.72	0.994
Chardonnay B	2005-06	Distal	-	0.046	5.70	0.987
Chardonnay B	2005-06	Distal	-	0.044	4.25	0.980
Chardonnay B	2005-06	Distal	-	0.041	5.05	0.992
Chardonnay B	2005-06	Medial	-	0.029	3.50	0.988
Chardonnay B	2005-06	Medial	-	0.035	2.64	0.984
Chardonnay B	2005-06	Medial	-	0.041	4.42	0.991
Chardonnay B	2005-06	Medial	-	0.044	3.84	0.980
Chardonnay B	2005-06	Medial	-	0.031	3.21	0.991
Chardonnay B	2005-06	Medial	-	0.033	-0.14	0.986
Chardonnay B	2005-06	Medial	-	0.035	3.05	0.998
Chardonnay B	2005-06	Medial	-	0.037	2.78	0.996
Chardonnay B	2005-06	Medial	-	0.023	3.84	0.985
Chardonnay B	2005-06	Medial	-	0.045	3.64	0.995
Chardonnay B	2005-06	Medial	-	0.045	2.69	0.997
Chardonnay B	2005-06	Medial	-	0.043	2.85	0.994
Chardonnay B	2005-06	Basal	-	0.033	5.28	0.976
Chardonnay B	2005-06	Basal	-	0.045	1.46	0.996
Chardonnay B	2005-06	Basal	-	0.045	2.80	0.991
Chardonnay B	2005-06	Basal	-	0.042	2.78	0.996
Chardonnay B	2005-06	Basal	-	0.048	3.34	0.996
Chardonnay B	2005-06	Basal	-	0.046	0.86	0.996
Chardonnay B	2005-06	Basal	-	0.038	4.25	0.978
Chardonnay B	2005-06	Basal	-	0.045	4.07	0.975
Chardonnay B	2005-06	Basal	-	0.041	4.15	0.992
Chardonnay B	2005-06	Basal	-	0.039	1.49	0.991
Chardonnay B	2005-06	Basal	-	0.052	1.52	0.998

Chardonnay B	2005-06	Basal	-	0.042	3.62	0.989
Chardonnay B	2006-07	Distal	-	0.025	2.41	0.989
Chardonnay B	2006-07	Distal	-	0.037	3.78	0.989
Chardonnay B	2006-07	Distal	-	0.058	1.09	0.987
Chardonnay B	2006-07	Distal	-	0.044	2.48	0.994
Chardonnay B	2006-07	Distal	-	0.026	2.26	0.992
Chardonnay B	2006-07	Distal	-	0.035	3.81	0.973
Chardonnay B	2006-07	Distal	-	0.047	3.10	0.968
Chardonnay B	2006-07	Distal	-	0.035	4.13	0.951
Chardonnay B	2006-07	Distal	-	0.039	3.10	0.971
Chardonnay B	2006-07	Distal	-	0.042	4.20	0.994
Chardonnay B	2006-07	Distal	-	0.038	1.78	0.985
Chardonnay B	2006-07	Distal	-	0.032	2.39	0.968
Chardonnay B	2006-07	Medial	-	0.023	1.65	0.960
Chardonnay B	2006-07	Medial	-	0.035	2.76	0.976
Chardonnay B	2006-07	Medial	-	0.041	2.01	0.980
Chardonnay B	2006-07	Medial	-	0.039	1.88	0.982
Chardonnay B	2006-07	Medial	-	0.041	0.81	0.975
Chardonnay B	2006-07	Medial	-	0.028	0.90	0.977
Chardonnay B	2006-07	Medial	-	0.033	3.26	0.965
Chardonnay B	2006-07	Medial	-	0.024	1.67	0.969
Chardonnay B	2006-07	Medial	-	0.034	2.08	0.965
Chardonnay B	2006-07	Medial	-	0.023	1.14	0.995
Chardonnay B	2006-07	Medial	-	0.022	2.50	0.970
Chardonnay B	2006-07	Basal	-	0.031	1.51	0.974
Chardonnay B	2006-07	Basal	-	0.041	2.25	0.991
Chardonnay B	2006-07	Basal	-	0.047	1.91	0.997
Chardonnay B	2006-07	Basal	-	0.052	0.77	0.974
Chardonnay B	2006-07	Basal	-	0.046	0.69	0.996
Chardonnay B	2006-07	Basal	-	0.031	2.43	0.985
Chardonnay B	2006-07	Basal	-	0.030	0.44	0.959
Chardonnay B	2006-07	Basal	-	0.038	0.73	0.962
Chardonnay B	2006-07	Basal	-	0.046	0.59	0.960
Chardonnay B	2006-07	Basal	-	0.056	0.08	0.981
Chardonnay B	2006-07	Basal	-	0.044	1.34	0.984
Pinot noir A	2005-06	Distal	Upwards	0.036	3.60	0.999
Pinot noir A	2005-06	Distal	Downwards	0.043	3.17	0.981
Pinot noir A	2005-06	Distal	Upwards	0.036	3.78	0.997
Pinot noir A	2005-06	Distal	Downwards	0.025	0.66	0.994
Pinot noir A	2005-06	Distal	Upwards	0.035	2.87	0.997
Pinot noir A	2005-06	Distal	Downwards	0.039	1.81	0.993
Pinot noir A	2005-06	Distal	Upwards	0.039	3.53	0.989
Pinot noir A	2005-06	Distal	Downwards	0.043	1.63	0.988
Pinot noir A	2005-06	Distal	Upwards	0.035	4.05	0.996
Pinot noir A	2005-06	Distal	Downwards	0.038	2.21	0.996
Pinot noir A	2005-06	Distal	Upwards	0.035	5.47	0.990
Pinot noir A	2005-06	Distal	Downwards	0.037	3.41	0.998
Pinot noir A	2005-06	Medial	Upwards	0.031	4.54	0.998
Pinot noir A	2005-06	Medial	Downwards	0.035	3.97	0.999
Pinot noir A	2005-06	Medial	Upwards	0.034	2.85	0.990
Pinot noir A	2005-06	Medial	Downwards	0.040	2.45	0.992
Pinot noir A	2005-06	Medial	Upwards	0.038	2.53	0.989
Pinot noir A	2005-06	Medial	Downwards	0.038	0.20	0.996
Pinot noir A	2005-06	Medial	Upwards	0.027	4.72	0.986
Pinot noir A	2005-06	Medial	Downwards	0.043	1.23	0.994
Pinot noir A	2005-06	Medial	Upwards	0.037	3.91	0.996
Pinot noir A	2005-06	Medial	Downwards	0.035	3.82	0.986
Pinot noir A	2005-06	Medial	Upwards	0.040	3.97	0.994

Pinot noir A	2005-06	Medial	Downwards	0.035	3.34	0.994
Pinot noir A	2005-06	Basal	Upwards	0.040	2.79	0.994
Pinot noir A	2005-06	Basal	Upwards	0.041	1.46	0.969
Pinot noir A	2005-06	Basal	Downwards	0.041	3.51	0.992
Pinot noir A	2005-06	Basal	Upwards	0.034	3.14	0.984
Pinot noir A	2005-06	Basal	Downwards	0.037	1.43	0.996
Pinot noir A	2005-06	Basal	Upwards	0.041	1.61	0.995
Pinot noir A	2005-06	Basal	Downwards	0.044	1.53	0.995
Pinot noir A	2005-06	Basal	Upwards	0.038	2.04	0.996
Pinot noir A	2005-06	Basal	Upwards	0.038	1.76	0.994
Pinot noir A	2005-06	Distal	Upwards	0.039	1.81	0.979
Pinot noir A	2006-07	Distal	Downwards	0.043	0.57	0.989
Pinot noir A	2006-07	Distal	Upwards	0.045	1.11	0.983
Pinot noir A	2006-07	Distal	Downwards	0.054	0.36	0.997
Pinot noir A	2006-07	Distal	Upwards	0.041	1.85	0.972
Pinot noir A	2006-07	Distal	Downwards	0.036	2.02	0.989
Pinot noir A	2006-07	Distal	Upwards	0.036	2.94	0.994
Pinot noir A	2006-07	Distal	Downwards	0.026	1.99	0.987
Pinot noir A	2006-07	Distal	Upwards	0.037	1.65	0.991
Pinot noir A	2006-07	Distal	Downwards	0.035	1.30	0.959
Pinot noir A	2006-07	Distal	Upwards	0.044	2.08	0.967
Pinot noir A	2006-07	Distal	Downwards	0.037	2.78	0.991
Pinot noir A	2006-07	Medial	Upwards	0.037	1.46	0.994
Pinot noir A	2006-07	Medial	Downwards	0.049	0.44	0.992
Pinot noir A	2006-07	Medial	Upwards	0.039	0.52	0.990
Pinot noir A	2006-07	Medial	Downwards	0.049	0.97	0.991
Pinot noir A	2006-07	Medial	Upwards	0.025	1.87	0.986
Pinot noir A	2006-07	Medial	Upwards	0.036	2.10	0.981
Pinot noir A	2006-07	Medial	Downwards	0.034	-0.04	0.999
Pinot noir A	2006-07	Medial	Upwards	0.033	2.45	0.995
Pinot noir A	2006-07	Medial	Downwards	0.040	1.52	0.989
Pinot noir A	2006-07	Medial	Upwards	0.028	2.12	0.988
Pinot noir A	2006-07	Medial	Downwards	0.036	1.59	0.994
Pinot noir A	2006-07	Basal	Upwards	0.034	2.45	0.989
Pinot noir A	2006-07	Basal	Upwards	0.041	2.64	0.931
Pinot noir A	2006-07	Basal	Downwards	0.050	0.51	0.993
Pinot noir A	2006-07	Basal	Upwards	0.039	1.88	0.993
Pinot noir A	2006-07	Basal	Downwards	0.027	2.85	0.989
Pinot noir A	2006-07	Basal	Upwards	0.039	1.66	0.993
Pinot noir A	2006-07	Basal	Upwards	0.025	0.68	0.985
Pinot noir A	2006-07	Basal	Downwards	0.038	1.68	0.993
Pinot noir A	2006-07	Basal	Upwards	0.029	2.44	0.965
Pinot noir A	2006-07	Basal	Downwards	0.033	3.14	0.992
Pinot noir B	2005-06	Distal	Upwards	0.035	4.01	0.996
Pinot noir B	2005-06	Distal	Downwards	0.036	4.16	0.990
Pinot noir B	2005-06	Distal	Upwards	0.042	3.09	0.996
Pinot noir B	2005-06	Distal	Downwards	0.033	4.05	0.993
Pinot noir B	2005-06	Distal	Downwards	0.037	5.27	0.990
Pinot noir B	2005-06	Distal	Upwards	0.035	3.99	0.988
Pinot noir B	2005-06	Distal	Downwards	0.045	2.53	0.993
Pinot noir B	2005-06	Distal	Upwards	0.037	2.18	0.999
Pinot noir B	2005-06	Distal	Downwards	0.034	3.88	0.974
Pinot noir B	2005-06	Distal	Upwards	0.041	2.82	0.993
Pinot noir B	2005-06	Distal	Downwards	0.036	3.27	0.992
Pinot noir B	2005-06	Medial	Upwards	0.027	2.60	0.999
Pinot noir B	2005-06	Medial	Downwards	0.036	4.10	0.977
Pinot noir B	2005-06	Medial	Upwards	0.031	3.80	0.980
Pinot noir B	2005-06	Medial	Downwards	0.035	3.34	0.995

Pinot noir B	2005-06	Medial	Upwards	0.021	3.11	0.986
Pinot noir B	2005-06	Medial	Downwards	0.043	1.67	0.986
Pinot noir B	2005-06	Medial	Upwards	0.037	4.46	0.997
Pinot noir B	2005-06	Medial	Downwards	0.036	3.41	0.984
Pinot noir B	2005-06	Medial	Upwards	0.027	2.32	0.961
Pinot noir B	2005-06	Medial	Downwards	0.030	4.18	0.973
Pinot noir B	2005-06	Medial	Upwards	0.041	1.82	0.996
Pinot noir B	2005-06	Medial	Downwards	0.039	2.36	0.968
Pinot noir B	2005-06	Basal	Upwards	0.029	2.93	0.973
Pinot noir B	2005-06	Basal	Downwards	0.037	3.65	0.992
Pinot noir B	2005-06	Basal	Upwards	0.031	2.88	0.983
Pinot noir B	2005-06	Basal	Downwards	0.041	3.00	0.943
Pinot noir B	2005-06	Basal	Upwards	0.034	2.52	0.990
Pinot noir B	2005-06	Basal	Downwards	0.032	5.15	0.979
Pinot noir B	2005-06	Basal	Upwards	0.036	2.96	0.995
Pinot noir B	2005-06	Basal	Downwards	0.035	3.42	0.983
Pinot noir B	2005-06	Basal	Upwards	0.038	2.65	0.993
Pinot noir B	2005-06	Basal	Downwards	0.024	3.82	0.972
Pinot noir B	2005-06	Basal	Upwards	0.040	2.57	0.991
Pinot noir B	2005-06	Basal	Downwards	0.045	2.06	0.989
Pinot noir B	2006-07	Distal	Upwards	0.031	4.04	0.994
Pinot noir B	2006-07	Distal	Downwards	0.027	3.45	0.994
Pinot noir B	2006-07	Distal	Upwards	0.039	1.87	0.999
Pinot noir B	2006-07	Distal	Downwards	0.040	2.60	0.962
Pinot noir B	2006-07	Distal	Upwards	0.040	1.48	0.983
Pinot noir B	2006-07	Distal	Downwards	0.033	4.03	0.991
Pinot noir B	2006-07	Distal	Upwards	0.040	2.83	0.999
Pinot noir B	2006-07	Distal	Downwards	0.044	3.61	0.993
Pinot noir B	2006-07	Distal	Upwards	0.048	1.34	0.992
Pinot noir B	2006-07	Distal	Downwards	0.034	2.60	0.990
Pinot noir B	2006-07	Distal	Upwards	0.038	2.31	0.994
Pinot noir B	2006-07	Distal	Downwards	0.036	3.33	0.996
Pinot noir B	2006-07	Medial	Upwards	0.030	1.06	0.900
Pinot noir B	2006-07	Medial	Downwards	0.014	3.11	0.988
Pinot noir B	2006-07	Medial	Upwards	0.024	3.44	0.992
Pinot noir B	2006-07	Medial	Downwards	0.025	-0.32	0.999
Pinot noir B	2006-07	Medial	Upwards	0.030	3.11	0.975
Pinot noir B	2006-07	Medial	Downwards	0.039	2.79	0.992
Pinot noir B	2006-07	Medial	Upwards	0.022	-0.35	0.991
Pinot noir B	2006-07	Medial	Downwards	0.030	1.77	0.978
Pinot noir B	2006-07	Medial	Upwards	0.027	0.34	0.998
Pinot noir B	2006-07	Basal	Upwards	0.030	3.53	0.989
Pinot noir B	2006-07	Basal	Upwards	0.047	0.88	0.993
Pinot noir B	2006-07	Basal	Downwards	0.021	0.17	0.953
Pinot noir B	2006-07	Basal	Downwards	0.034	2.64	0.997
Pinot noir B	2006-07	Basal	Upwards	0.028	2.41	0.961
Pinot noir B	2006-07	Basal	Upwards	0.037	1.16	0.988
Pinot noir B	2006-07	Basal	Downwards	0.024	1.56	0.995
Pinot noir B	2006-07	Basal	Upwards	0.033	3.05	0.939
Shoot length						
Block	**Season**	**Shoot**	**Shoot orientation**	**Slope**	**Intercept**	**R^2**
Chardonnay A	2005-06	Distal	-	5.26	-50.63	0.995
Chardonnay A	2005-06	Distal	-	4.37	33.52	0.987
Chardonnay A	2005-06	Distal	-	5.42	-194.78	0.991
Chardonnay A	2005-06	Distal	-	3.47	59.46	0.999
Chardonnay A	2005-06	Distal	-	5.67	-55.82	0.995
Chardonnay A	2005-06	Distal	-	2.62	71.99	0.994
Chardonnay A	2005-06	Distal	-	5.33	34.42	0.997

Chardonnay A	2005-06	Distal	-	4.37	33.52	0.987
Chardonnay A	2005-06	Distal	-	3.33	118.85	0.964
Chardonnay A	2005-06	Distal	-	4.56	-21.39	0.994
Chardonnay A	2005-06	Distal	-	4.76	-83.38	0.990
Chardonnay A	2005-06	Medial	-	1.75	-52.73	0.964
Chardonnay A	2005-06	Medial	-	2.46	-49.11	0.989
Chardonnay A	2005-06	Medial	-	3.08	-73.10	0.989
Chardonnay A	2005-06	Medial	-	3.81	-96.20	0.986
Chardonnay A	2005-06	Medial	-	3.40	-136.28	0.981
Chardonnay A	2005-06	Medial	-	3.22	-157.71	0.986
Chardonnay A	2005-06	Medial	-	1.32	33.03	0.973
Chardonnay A	2005-06	Medial	-	2.27	13.08	0.990
Chardonnay A	2005-06	Medial	-	1.99	-43.54	0.990
Chardonnay A	2005-06	Medial	-	1.03	49.37	0.947
Chardonnay A	2005-06	Medial	-	3.46	-152.60	0.991
Chardonnay A	2005-06	Basal	-	5.00	21.37	0.997
Chardonnay A	2005-06	Basal	-	3.96	37.11	0.989
Chardonnay A	2005-06	Basal	-	3.82	-94.18	0.993
Chardonnay A	2005-06	Basal	-	3.23	-119.76	0.964
Chardonnay A	2005-06	Basal	-	5.23	24.23	0.988
Chardonnay A	2005-06	Basal	-	2.68	18.89	0.974
Chardonnay A	2005-06	Basal	-	3.54	29.55	0.991
Chardonnay A	2005-06	Basal	-	3.91	-126.28	0.995
Chardonnay A	2005-06	Basal	-	5.19	-126.84	0.996
Chardonnay A	2005-06	Basal	-	2.86	-48.76	0.996
Chardonnay A	2005-06	Basal	-	3.59	-175.39	0.993
Chardonnay A	2006-07	Distal	-	2.44	-12.01	0.982
Chardonnay A	2006-07	Distal	-	3.40	60.06	0.989
Chardonnay A	2006-07	Distal	-	2.73	-23.51	0.991
Chardonnay A	2006-07	Distal	-	2.88	40.05	0.984
Chardonnay A	2006-07	Distal	-	1.85	19.20	0.992
Chardonnay A	2006-07	Distal	-	2.80	82.90	0.975
Chardonnay A	2006-07	Distal	-	2.90	-72.18	0.995
Chardonnay A	2006-07	Distal	-	3.19	-132.78	0.995
Chardonnay A	2006-07	Distal	-	1.84	66.57	0.994
Chardonnay A	2006-07	Medial	-	0.93	-5.43	0.986
Chardonnay A	2006-07	Medial	-	2.37	-56.22	0.992
Chardonnay A	2006-07	Medial	-	1.20	15.76	0.969
Chardonnay A	2006-07	Medial	-	2.57	40.99	0.970
Chardonnay A	2006-07	Medial	-	0.77	55.34	0.975
Chardonnay A	2006-07	Medial	-	0.30	77.52	0.895
Chardonnay A	2006-07	Medial	-	1.62	26.29	0.991
Chardonnay A	2006-07	Medial	-	1.79	-41.02	0.956
Chardonnay A	2006-07	Medial	-	0.75	37.70	0.993
Chardonnay A	2006-07	Basal	-	3.12	-109.14	0.992
Chardonnay A	2006-07	Basal	-	4.20	-242.99	0.995
Chardonnay A	2006-07	Basal	-	2.98	37.77	0.953
Chardonnay A	2006-07	Basal	-	2.96	-50.05	0.994
Chardonnay A	2006-07	Basal	-	3.32	-41.67	0.987
Chardonnay A	2006-07	Basal	-	2.68	-13.48	0.991
Chardonnay A	2006-07	Basal	-	3.18	-43.45	0.994
Chardonnay A	2006-07	Basal	-	3.25	-17.29	0.983
Chardonnay B	2005-06	Distal	-	3.13	114.67	0.997
Chardonnay B	2005-06	Distal	-	2.79	190.19	0.995
Chardonnay B	2005-06	Distal	-	2.80	159.91	0.986
Chardonnay B	2005-06	Distal	-	0.83	96.35	0.971
Chardonnay B	2005-06	Distal	-	3.32	75.88	0.990
Chardonnay B	2005-06	Distal	-	3.02	110.50	0.993

Chardonnay B	2005-06	Distal	-	2.17	103.91	0.990
Chardonnay B	2005-06	Distal	-	1.32	133.37	0.973
Chardonnay B	2005-06	Distal	-	2.62	147.26	0.990
Chardonnay B	2005-06	Distal	-	3.02	287.38	0.983
Chardonnay B	2005-06	Distal	-	2.75	205.60	0.988
Chardonnay B	2005-06	Distal	-	2.70	246.16	0.977
Chardonnay B	2005-06	Medial	-	1.11	118.50	0.965
Chardonnay B	2005-06	Medial	-	1.37	67.02	0.985
Chardonnay B	2005-06	Medial	-	2.37	118.29	0.996
Chardonnay B	2005-06	Medial	-	0.97	62.14	0.982
Chardonnay B	2005-06	Medial	-	0.66	126.96	0.917
Chardonnay B	2005-06	Medial	-	1.50	92.15	0.986
Chardonnay B	2005-06	Medial	-	1.76	52.01	0.996
Chardonnay B	2005-06	Medial	-	0.74	97.58	0.959
Chardonnay B	2005-06	Medial	-	1.95	78.08	0.999
Chardonnay B	2005-06	Medial	-	2.65	29.32	0.991
Chardonnay B	2005-06	Basal	-	2.72	6.12	0.993
Chardonnay B	2005-06	Basal	-	1.63	170.77	0.982
Chardonnay B	2005-06	Basal	-	2.77	-19.87	0.979
Chardonnay B	2005-06	Basal	-	3.42	39.42	0.998
Chardonnay B	2005-06	Basal	-	2.72	36.08	0.988
Chardonnay B	2005-06	Basal	-	3.16	130.02	0.994
Chardonnay B	2005-06	Basal	-	2.84	-15.94	0.992
Chardonnay B	2005-06	Basal	-	2.47	212.46	0.983
Chardonnay B	2005-06	Basal	-	2.89	138.74	0.994
Chardonnay B	2005-06	Basal	-	2.44	222.61	0.964
Chardonnay B	2005-06	Basal	-	1.39	135.61	0.925
Chardonnay B	2005-06	Basal	-	3.44	-11.71	0.996
Chardonnay B	2005-06	Basal	-	2.51	76.60	0.991
Chardonnay B	2006-07	Distal	-	0.72	74.57	0.984
Chardonnay B	2006-07	Distal	-	2.21	91.44	0.964
Chardonnay B	2006-07	Distal	-	2.49	34.24	0.961
Chardonnay B	2006-07	Distal	-	1.43	97.45	0.986
Chardonnay B	2006-07	Distal	-	0.56	76.96	0.968
Chardonnay B	2006-07	Distal	-	1.52	47.34	0.940
Chardonnay B	2006-07	Distal	-	1.71	105.76	0.897
Chardonnay B	2006-07	Distal	-	1.76	37.75	0.954
Chardonnay B	2006-07	Distal	-	1.48	54.78	0.933
Chardonnay B	2006-07	Distal	-	1.48	181.94	0.995
Chardonnay B	2006-07	Distal	-	2.89	67.37	0.927
Chardonnay B	2006-07	Distal	-	1.75	51.13	0.984
Chardonnay B	2006-07	Medial	-	1.16	49.17	0.973
Chardonnay B	2006-07	Medial	-	1.40	23.96	0.972
Chardonnay B	2006-07	Medial	-	1.70	63.77	0.939
Chardonnay B	2006-07	Medial	-	1.01	99.99	0.969
Chardonnay B	2006-07	Medial	-	1.14	94.10	0.921
Chardonnay B	2006-07	Medial	-	0.39	35.78	0.965
Chardonnay B	2006-07	Medial	-	0.90	145.71	0.945
Chardonnay B	2006-07	Medial	-	0.34	49.81	0.981
Chardonnay B	2006-07	Medial	-	0.94	104.34	0.960
Chardonnay B	2006-07	Medial	-	0.23	49.87	0.946
Chardonnay B	2006-07	Medial	-	0.64	50.35	0.922
Chardonnay B	2006-07	Basal	-	1.29	-20.27	0.948
Chardonnay B	2006-07	Basal	-	1.59	22.57	0.975
Chardonnay B	2006-07	Basal	-	2.92	-17.11	0.984
Chardonnay B	2006-07	Basal	-	2.55	-3.00	0.979
Chardonnay B	2006-07	Basal	-	0.84	39.97	0.980
Chardonnay B	2006-07	Basal	-	1.03	106.16	0.971

Chardonnay B	2006-07	Basal	-	0.49	33.28	0.958
Chardonnay B	2006-07	Basal	-	1.06	28.41	0.897
Chardonnay B	2006-07	Basal	-	1.45	-16.64	0.975
Chardonnay B	2006-07	Basal	-	2.50	31.10	0.932
Chardonnay B	2006-07	Basal	-	2.35	-3.03	0.980
Pinot noir A	2005-06	Distal	Upwards	5.99	-201.10	0.979
Pinot noir A	2005-06	Distal	Downwards	5.40	-92.53	0.985
Pinot noir A	2005-06	Distal	Upwards	3.05	48.37	0.997
Pinot noir A	2005-06	Distal	Upwards	2.82	46.71	0.974
Pinot noir A	2005-06	Distal	Downwards	4.24	-98.23	0.997
Pinot noir A	2005-06	Distal	Upwards	4.62	-49.90	0.998
Pinot noir A	2005-06	Distal	Downwards	4.68	-182.96	0.971
Pinot noir A	2005-06	Distal	Upwards	4.08	52.74	0.986
Pinot noir A	2005-06	Distal	Downwards	3.56	-2.23	0.999
Pinot noir A	2005-06	Distal	Upwards	3.49	150.85	0.992
Pinot noir A	2005-06	Distal	Downwards	2.95	41.25	0.997
Pinot noir A	2005-06	Medial	Upwards	3.11	98.80	0.996
Pinot noir A	2005-06	Medial	Downwards	3.68	182.39	0.931
Pinot noir A	2005-06	Medial	Upwards	2.10	42.59	0.993
Pinot noir A	2005-06	Medial	Downwards	3.29	3.63	0.994
Pinot noir A	2005-06	Medial	Upwards	3.61	80.88	0.981
Pinot noir A	2005-06	Medial	Downwards	2.98	-81.84	0.995
Pinot noir A	2005-06	Medial	Upwards	2.36	165.40	0.968
Pinot noir A	2005-06	Medial	Downwards	4.14	-114.10	0.987
Pinot noir A	2005-06	Medial	Upwards	3.74	1.08	0.998
Pinot noir A	2005-06	Medial	Downwards	3.52	53.80	0.971
Pinot noir A	2005-06	Medial	Upwards	3.95	52.97	1.000
Pinot noir A	2005-06	Medial	Downwards	2.93	27.42	0.994
Pinot noir A	2005-06	Basal	Upwards	4.48	-101.17	0.998
Pinot noir A	2005-06	Basal	Downwards	3.65	163.15	0.999
Pinot noir A	2005-06	Basal	Upwards	2.21	36.99	0.996
Pinot noir A	2005-06	Basal	Downwards	4.73	12.48	0.997
Pinot noir A	2005-06	Basal	Upwards	2.88	55.97	0.969
Pinot noir A	2005-06	Basal	Downwards	4.22	-145.15	0.997
Pinot noir A	2005-06	Basal	Upwards	3.38	-75.62	0.996
Pinot noir A	2005-06	Basal	Downwards	4.95	-185.93	0.998
Pinot noir A	2005-06	Basal	Upwards	3.47	-23.90	0.993
Pinot noir A	2005-06	Basal	Downwards	4.10	-22.21	0.991
Pinot noir A	2005-06	Basal	Upwards	2.90	-30.45	0.997
Pinot noir A	2005-06	Basal	Downwards	3.22	60.25	0.994
Pinot noir A	2006-07	Distal	Upwards	4.84	-203.34	0.999
Pinot noir A	2006-07	Distal	Downwards	3.52	-102.65	0.992
Pinot noir A	2006-07	Distal	Upwards	3.76	-43.92	0.949
Pinot noir A	2006-07	Distal	Downwards	4.38	-124.12	0.994
Pinot noir A	2006-07	Distal	Upwards	3.93	-65.45	0.959
Pinot noir A	2006-07	Distal	Downwards	2.76	-67.67	0.983
Pinot noir A	2006-07	Distal	Upwards	3.78	5.95	0.971
Pinot noir A	2006-07	Distal	Downwards	1.28	-24.25	0.976
Pinot noir A	2006-07	Distal	Upwards	2.45	-31.71	0.985
Pinot noir A	2006-07	Distal	Upwards	3.96	-53.48	0.983
Pinot noir A	2006-07	Distal	Downwards	3.20	-37.65	0.990
Pinot noir A	2006-07	Medial	Upwards	3.07	-72.54	0.997
Pinot noir A	2006-07	Medial	Downwards	4.79	-136.83	0.979
Pinot noir A	2006-07	Medial	Upwards	1.74	-0.15	0.972
Pinot noir A	2006-07	Medial	Downwards	3.13	-10.05	0.949
Pinot noir A	2006-07	Medial	Upwards	1.30	5.28	0.981
Pinot noir A	2006-07	Medial	Upwards	3.11	-77.96	0.984
Pinot noir A	2006-07	Medial	Downwards	1.51	-106.40	0.984

Pinot noir A	2006-07	Medial	Upwards	2.50	-3.70	0.981
Pinot noir A	2006-07	Medial	Downwards	2.33	24.42	0.958
Pinot noir A	2006-07	Medial	Upwards	1.29	35.78	0.986
Pinot noir A	2006-07	Medial	Downwards	2.07	-13.15	0.983
Pinot noir A	2006-07	Basal	Upwards	2.63	-26.97	0.996
Pinot noir A	2006-07	Basal	Upwards	4.04	-129.29	0.999
Pinot noir A	2006-07	Basal	Downwards	3.43	-162.45	0.985
Pinot noir A	2006-07	Basal	Upwards	3.30	-106.38	0.979
Pinot noir A	2006-07	Basal	Upwards	2.78	-59.04	0.988
Pinot noir A	2006-07	Basal	Downwards	3.41	-50.05	0.997
Pinot noir A	2006-07	Basal	Upwards	1.97	12.80	0.967
Pinot noir A	2006-07	Basal	Downwards	3.21	-2.67	0.981
Pinot noir B	2005-06	Distal	Upwards	2.41	142.30	0.980
Pinot noir B	2005-06	Distal	Downwards	2.31	243.44	0.937
Pinot noir B	2005-06	Distal	Upwards	2.72	97.35	0.986
Pinot noir B	2005-06	Distal	Downwards	2.05	188.87	0.911
Pinot noir B	2005-06	Distal	Upwards	3.58	77.80	0.918
Pinot noir B	2005-06	Distal	Downwards	2.50	217.12	0.978
Pinot noir B	2005-06	Distal	Upwards	2.05	294.18	0.936
Pinot noir B	2005-06	Distal	Downwards	4.03	60.62	0.953
Pinot noir B	2005-06	Distal	Upwards	2.16	82.13	0.976
Pinot noir B	2005-06	Distal	Downwards	2.62	95.92	0.977
Pinot noir B	2005-06	Distal	Upwards	3.12	137.71	0.978
Pinot noir B	2005-06	Distal	Downwards	2.69	141.96	0.976
Pinot noir B	2005-06	Medial	Upwards	1.21	64.83	0.993
Pinot noir B	2005-06	Medial	Downwards	1.85	145.83	0.979
Pinot noir B	2005-06	Medial	Upwards	1.50	95.38	0.985
Pinot noir B	2005-06	Medial	Downwards	1.90	133.46	0.951
Pinot noir B	2005-06	Medial	Upwards	0.93	78.51	0.973
Pinot noir B	2005-06	Medial	Downwards	1.55	126.87	0.915
Pinot noir B	2005-06	Medial	Upwards	3.29	178.33	0.986
Pinot noir B	2005-06	Medial	Downwards	1.40	292.93	0.889
Pinot noir B	2005-06	Medial	Upwards	1.03	57.83	0.928
Pinot noir B	2005-06	Medial	Downwards	1.74	104.57	0.981
Pinot noir B	2005-06	Medial	Upwards	2.45	62.18	0.967
Pinot noir B	2005-06	Medial	Downwards	2.62	52.13	0.954
Pinot noir B	2005-06	Basal	Upwards	0.59	121.51	0.941
Pinot noir B	2005-06	Basal	Downwards	2.05	54.79	0.998
Pinot noir B	2005-06	Basal	Upwards	1.62	52.19	0.990
Pinot noir B	2005-06	Basal	Downwards	3.19	-25.96	0.995
Pinot noir B	2005-06	Basal	Upwards	2.83	0.70	0.992
Pinot noir B	2005-06	Basal	Downwards	4.60	-118.54	0.979
Pinot noir B	2005-06	Basal	Upwards	3.67	31.66	0.987
Pinot noir B	2005-06	Basal	Downwards	2.01	192.71	0.865
Pinot noir B	2005-06	Basal	Upwards	2.98	33.38	0.986
Pinot noir B	2005-06	Basal	Downwards	1.25	80.75	0.928
Pinot noir B	2005-06	Basal	Upwards	4.26	-72.92	0.996
Pinot noir B	2005-06	Basal	Downwards	4.03	-18.08	0.986
Pinot noir B	2006-07	Distal	Upwards	1.11	153.90	0.956
Pinot noir B	2006-07	Distal	Downwards	0.70	159.99	0.923
Pinot noir B	2006-07	Distal	Upwards	1.63	55.27	0.993
Pinot noir B	2006-07	Distal	Downwards	2.62	-33.50	0.986
Pinot noir B	2006-07	Distal	Upwards	2.30	40.90	0.908
Pinot noir B	2006-07	Distal	Downwards	2.26	64.62	0.968
Pinot noir B	2006-07	Distal	Upwards	3.14	-58.22	0.991
Pinot noir B	2006-07	Distal	Downwards	2.94	73.49	0.959
Pinot noir B	2006-07	Distal	Upwards	2.29	0.66	0.937
Pinot noir B	2006-07	Distal	Downwards	1.17	72.30	0.951

Pinot noir B	2006-07	Distal	Upwards	1.53	54.42	0.983
Pinot noir B	2006-07	Distal	Downwards	1.82	-10.57	0.991
Pinot noir B	2006-07	Medial	Upwards	0.18	41.05	0.902
Pinot noir B	2006-07	Medial	Downwards	0.45	51.34	0.949
Pinot noir B	2006-07	Medial	Downwards	0.24	56.25	0.952
Pinot noir B	2006-07	Medial	Upwards	0.79	116.19	0.897
Pinot noir B	2006-07	Medial	Downwards	0.69	-40.85	0.963
Pinot noir B	2006-07	Medial	Upwards	1.61	14.58	0.935
Pinot noir B	2006-07	Medial	Downwards	2.28	21.21	0.984
Pinot noir B	2006-07	Medial	Downwards	1.10	27.74	0.983
Pinot noir B	2006-07	Medial	Upwards	0.59	3.73	0.992
Pinot noir B	2006-07	Basal	Upwards	1.25	97.09	0.887
Pinot noir B	2006-07	Basal	Downwards	0.52	38.30	0.849
Pinot noir B	2006-07	Basal	Upwards	2.09	58.25	0.977
Pinot noir B	2006-07	Basal	Downwards	0.23	37.58	0.874
Pinot noir B	2006-07	Basal	Upwards	1.56	98.25	0.949
Pinot noir B	2006-07	Basal	Downwards	0.91	102.22	0.831
Pinot noir B	2006-07	Basal	Upwards	0.70	114.34	0.940
Pinot noir B	2006-07	Basal	Downwards	0.64	33.78	0.990
Pinot noir B	2006-07	Basal	Upwards	3.42	-24.81	0.957
Pinot noir B	2006-07	Basal	Downwards	2.71	-84.52	0.995
Leaf area						
Block	**Season**	**Shoot**	**Shoot orientation**	**Slope**	**intercept**	**R^2**
Chardonnay A	2005-06	Distal	-	7.89	-358.56	0.993
Chardonnay A	2005-06	Distal	-	7.12	-174.59	0.996
Chardonnay A	2005-06	Distal	-	6.53	-353.75	0.989
Chardonnay A	2005-06	Distal	-	6.56	-198.02	0.993
Chardonnay A	2005-06	Distal	-	8.22	-305.99	0.995
Chardonnay A	2005-06	Distal	-	3.31	-23.79	0.963
Chardonnay A	2005-06	Distal	-	8.57	-386.43	0.994
Chardonnay A	2005-06	Distal	-	6.22	-272.65	0.986
Chardonnay A	2005-06	Distal	-	9.35	-271.63	1.000
Chardonnay A	2005-06	Distal	-	5.59	-80.55	0.998
Chardonnay A	2005-06	Distal	-	7.24	-283.44	0.991
Chardonnay A	2005-06	Distal	-	7.24	-372.33	0.987
Chardonnay A	2005-06	Medial	-	3.12	-221.37	0.975
Chardonnay A	2005-06	Medial	-	4.33	-275.58	0.987
Chardonnay A	2005-06	Medial	-	4.42	-167.90	0.985
Chardonnay A	2005-06	Medial	-	6.71	-397.26	0.972
Chardonnay A	2005-06	Medial	-	6.52	-204.13	0.999
Chardonnay A	2005-06	Medial	-	2.48	-59.25	0.996
Chardonnay A	2005-06	Medial	-	2.86	-78.50	0.981
Chardonnay A	2005-06	Medial	-	1.65	-15.03	0.992
Chardonnay A	2005-06	Medial	-	3.31	-41.94	0.996
Chardonnay A	2005-06	Medial	-	3.11	-176.77	0.959
Chardonnay A	2005-06	Medial	-	1.77	-28.91	0.989
Chardonnay A	2005-06	Medial	-	4.45	-290.08	0.994
Chardonnay A	2005-06	Basal	-	9.07	-501.70	0.980
Chardonnay A	2005-06	Basal	-	6.82	-277.33	0.987
Chardonnay A	2005-06	Basal	-	5.96	-349.78	0.994
Chardonnay A	2005-06	Basal	-	5.44	-319.17	0.983
Chardonnay A	2005-06	Basal	-	6.98	-198.55	0.978
Chardonnay A	2005-06	Basal	-	5.01	-204.83	0.994
Chardonnay A	2005-06	Basal	-	3.58	-114.16	0.983
Chardonnay A	2005-06	Basal	-	5.86	-314.50	0.993
Chardonnay A	2005-06	Basal	-	6.60	-455.85	0.985
Chardonnay A	2005-06	Basal	-	8.23	-480.66	0.991
Chardonnay A	2005-06	Basal	-	5.37	-286.11	0.994

Chardonnay A	2005-06	Basal	-	4.77	-403.16	0.991
Chardonnay A	2006-07	Distal	-	3.04	-160.30	0.985
Chardonnay A	2006-07	Distal	-	4.10	-238.35	0.980
Chardonnay A	2006-07	Distal	-	5.83	-225.91	0.984
Chardonnay A	2006-07	Distal	-	2.96	-152.66	0.963
Chardonnay A	2006-07	Distal	-	3.90	-189.98	0.991
Chardonnay A	2006-07	Distal	-	5.11	-248.79	0.985
Chardonnay A	2006-07	Distal	-	3.27	-149.82	0.970
Chardonnay A	2006-07	Distal	-	4.69	-185.41	0.976
Chardonnay A	2006-07	Distal	-	4.73	-254.37	0.987
Chardonnay A	2006-07	Distal	-	4.89	-329.10	0.988
Chardonnay A	2006-07	Distal	-	3.12	-80.08	0.987
Chardonnay A	2006-07	Medial	-	1.57	-88.19	0.994
Chardonnay A	2006-07	Medial	-	4.10	-269.57	0.992
Chardonnay A	2006-07	Medial	-	1.62	-82.87	0.985
Chardonnay A	2006-07	Medial	-	1.83	-136.83	0.899
Chardonnay A	2006-07	Medial	-	4.51	-239.28	0.965
Chardonnay A	2006-07	Medial	-	1.51	-72.08	0.977
Chardonnay A	2006-07	Medial	-	0.72	11.04	0.936
Chardonnay A	2006-07	Medial	-	2.91	-104.51	0.968
Chardonnay A	2006-07	Medial	-	2.56	-234.33	0.981
Chardonnay A	2006-07	Medial	-	1.32	-68.51	0.984
Chardonnay A	2006-07	Basal	-	5.11	-345.86	0.912
Chardonnay A	2006-07	Basal	-	4.62	-306.44	0.990
Chardonnay A	2006-07	Basal	-	5.17	-348.41	0.983
Chardonnay A	2006-07	Basal	-	6.22	-440.36	0.980
Chardonnay A	2006-07	Basal	-	1.25	-30.93	0.948
Chardonnay A	2006-07	Basal	-	5.22	-346.86	0.993
Chardonnay A	2006-07	Basal	-	5.08	-322.65	0.973
Chardonnay A	2006-07	Basal	-	4.60	-239.17	0.986
Chardonnay A	2006-07	Basal	-	4.82	-218.79	0.978
Chardonnay A	2006-07	Basal	-	5.67	-377.33	0.990
Chardonnay B	2005-06	Distal	-	3.85	211.90	0.945
Chardonnay B	2005-06	Distal	-	2.79	164.52	0.931
Chardonnay B	2005-06	Distal	-	4.54	89.83	0.969
Chardonnay B	2005-06	Distal	-	3.94	-75.95	0.998
Chardonnay B	2005-06	Distal	-	3.92	-1.24	0.988
Chardonnay B	2005-06	Distal	-	4.56	72.90	0.978
Chardonnay B	2005-06	Distal	-	4.70	74.31	0.966
Chardonnay B	2005-06	Distal	-	1.55	65.15	0.963
Chardonnay B	2005-06	Distal	-	4.87	-81.33	0.993
Chardonnay B	2005-06	Distal	-	2.35	129.49	0.949
Chardonnay B	2005-06	Distal	-	5.19	162.28	0.970
Chardonnay B	2005-06	Distal	-	3.97	151.17	0.949
Chardonnay B	2005-06	Medial	-	2.03	57.09	0.953
Chardonnay B	2005-06	Medial	-	3.96	54.99	0.958
Chardonnay B	2005-06	Medial	-	1.65	-30.39	0.996
Chardonnay B	2005-06	Medial	-	2.53	12.76	0.978
Chardonnay B	2005-06	Medial	-	1.69	1.08	0.975
Chardonnay B	2005-06	Medial	-	4.59	-132.68	0.983
Chardonnay B	2005-06	Medial	-	2.31	-24.26	0.955
Chardonnay B	2005-06	Medial	-	5.40	-188.20	0.981
Chardonnay B	2005-06	Medial	-	1.16	59.10	0.971
Chardonnay B	2005-06	Medial	-	2.47	19.62	0.955
Chardonnay B	2005-06	Medial	-	4.06	-138.69	0.978
Chardonnay B	2005-06	Medial	-	4.36	-132.99	0.957
Chardonnay B	2005-06	Basal	-	3.48	-21.52	0.959
Chardonnay B	2005-06	Basal	-	6.27	-330.98	0.991

Chardonnay B	2005-06	Basal	-	5.37	-189.95	0.993
Chardonnay B	2005-06	Basal	-	4.43	-46.47	0.979
Chardonnay B	2005-06	Basal	-	4.37	-83.19	0.981
Chardonnay B	2005-06	Basal	-	5.45	-304.89	0.990
Chardonnay B	2005-06	Basal	-	4.13	-192.75	0.965
Chardonnay B	2005-06	Basal	-	4.18	-76.76	0.987
Chardonnay B	2005-06	Basal	-	4.36	-242.05	0.990
Chardonnay B	2005-06	Basal	-	5.40	-16.00	0.939
Chardonnay B	2005-06	Basal	-	2.78	-101.21	0.976
Chardonnay B	2005-06	Basal	-	4.17	10.58	0.945
Chardonnay B	2006-07	Distal	-	1.23	2.38	0.974
Chardonnay B	2006-07	Distal	-	3.61	-96.50	0.967
Chardonnay B	2006-07	Distal	-	3.28	-47.53	0.944
Chardonnay B	2006-07	Distal	-	2.10	-21.77	0.959
Chardonnay B	2006-07	Distal	-	2.50	-56.73	0.949
Chardonnay B	2006-07	Distal	-	3.55	-45.29	0.970
Chardonnay B	2006-07	Distal	-	2.55	-10.16	0.987
Chardonnay B	2006-07	Distal	-	2.28	-19.38	0.936
Chardonnay B	2006-07	Distal	-	2.54	-25.59	0.960
Chardonnay B	2006-07	Distal	-	4.97	-204.92	0.988
Chardonnay B	2006-07	Distal	-	1.48	-24.63	0.987
Chardonnay B	2006-07	Medial	-	0.78	-19.24	0.988
Chardonnay B	2006-07	Medial	-	2.77	-58.54	0.960
Chardonnay B	2006-07	Medial	-	2.15	-118.88	0.993
Chardonnay B	2006-07	Medial	-	0.19	-1.77	0.916
Chardonnay B	2006-07	Medial	-	0.52	16.24	0.960
Chardonnay B	2006-07	Medial	-	0.56	-10.18	0.977
Chardonnay B	2006-07	Medial	-	2.43	-73.70	0.980
Chardonnay B	2006-07	Medial	-	1.93	17.72	0.948
Chardonnay B	2006-07	Medial	-	0.77	-49.41	0.984
Chardonnay B	2006-07	Medial	-	2.03	-20.98	0.948
Chardonnay B	2006-07	Medial	-	2.16	-70.52	0.965
Chardonnay B	2006-07	Medial	-	0.69	55.91	0.970
Chardonnay B	2006-07	Basal	-	1.20	-52.77	0.964
Chardonnay B	2006-07	Basal	-	3.12	-21.84	0.984
Chardonnay B	2006-07	Basal	-	2.02	-50.11	0.985
Chardonnay B	2006-07	Basal	-	1.78	-78.10	0.981
Chardonnay B	2006-07	Basal	-	3.53	-170.16	0.978
Chardonnay B	2006-07	Basal	-	2.48	-100.05	0.977
Chardonnay B	2006-07	Basal	-	4.00	-170.85	0.983
Chardonnay B	2006-07	Basal	-	1.65	-124.01	0.982
Chardonnay B	2006-07	Basal	-	0.80	-25.59	0.980
Chardonnay B	2006-07	Basal	-	2.19	-148.91	0.982
Chardonnay B	2006-07	Basal	-	3.59	-176.61	0.981
Pinot noir A	2005-06	Distal	Upwards	4.75	-36.73	0.991
Pinot noir A	2005-06	Distal	Upwards	4.53	-30.88	0.990
Pinot noir A	2005-06	Distal	Upwards	4.21	-156.22	0.983
Pinot noir A	2005-06	Distal	Upwards	5.01	29.05	0.974
Pinot noir A	2005-06	Distal	Upwards	5.18	31.91	0.997
Pinot noir A	2005-06	Distal	Upwards	5.26	-7.96	0.994
Pinot noir A	2005-06	Distal	Downwards	6.29	-242.46	0.964
Pinot noir A	2005-06	Distal	Downwards	4.84	-157.29	0.991
Pinot noir A	2005-06	Distal	Downwards	5.41	-294.79	0.975
Pinot noir A	2005-06	Distal	Downwards	4.13	-171.98	0.996
Pinot noir A	2005-06	Distal	Downwards	3.74	-27.23	0.994
Pinot noir A	2005-06	Medial	Upwards	4.41	-123.07	0.998
Pinot noir A	2005-06	Medial	Upwards	3.06	-58.18	0.997
Pinot noir A	2005-06	Medial	Upwards	4.58	-24.29	0.983

Pinot noir A	2005-06	Medial	Upwards	4.16	-77.74	0.987
Pinot noir A	2005-06	Medial	Upwards	5.04	-13.82	0.992
Pinot noir A	2005-06	Medial	Upwards	5.86	-123.01	0.990
Pinot noir A	2005-06	Medial	Downwards	5.80	-75.39	0.986
Pinot noir A	2005-06	Medial	Downwards	4.69	-125.73	0.996
Pinot noir A	2005-06	Medial	Downwards	3.20	-165.47	0.985
Pinot noir A	2005-06	Medial	Downwards	5.03	-335.91	0.983
Pinot noir A	2005-06	Medial	Downwards	4.81	-138.40	0.995
Pinot noir A	2005-06	Medial	Downwards	3.79	-31.87	0.998
Pinot noir A	2005-06	Basal	Upwards	3.72	-122.65	0.994
Pinot noir A	2005-06	Basal	Upwards	3.41	-121.66	0.906
Pinot noir A	2005-06	Basal	Upwards	4.96	-286.97	0.960
Pinot noir A	2005-06	Basal	Upwards	4.29	-239.79	0.997
Pinot noir A	2005-06	Basal	Upwards	4.69	-180.74	0.999
Pinot noir A	2005-06	Basal	Upwards	3.97	-190.75	0.988
Pinot noir A	2005-06	Basal	Downwards	5.87	-159.82	0.906
Pinot noir A	2005-06	Basal	Downwards	5.83	85.85	0.987
Pinot noir A	2005-06	Basal	Downwards	5.23	-367.76	0.992
Pinot noir A	2005-06	Basal	Downwards	6.65	-463.64	0.990
Pinot noir A	2005-06	Basal	Downwards	6.08	-192.52	0.993
Pinot noir A	2005-06	Basal	Downwards	4.94	-104.13	0.985
Pinot noir A	2005-06	Distal	Upwards	4.65	-257.71	0.984
Pinot noir A	2006-07	Distal	Upwards	4.48	-206.14	0.983
Pinot noir A	2006-07	Distal	Upwards	4.97	-208.06	0.987
Pinot noir A	2006-07	Distal	Upwards	5.17	-234.33	0.978
Pinot noir A	2006-07	Distal	Upwards	3.59	-179.62	0.976
Pinot noir A	2006-07	Distal	Upwards	5.88	-230.28	0.969
Pinot noir A	2006-07	Distal	Downwards	4.39	-295.52	0.992
Pinot noir A	2006-07	Distal	Downwards	4.99	-254.03	0.946
Pinot noir A	2006-07	Distal	Downwards	3.60	-207.61	0.985
Pinot noir A	2006-07	Distal	Downwards	2.40	-124.50	0.982
Pinot noir A	2006-07	Distal	Downwards	3.30	-87.48	0.994
Pinot noir A	2006-07	Distal	Downwards	3.73	-85.82	0.963
Pinot noir A	2006-07	Medial	Upwards	3.64	-160.57	0.966
Pinot noir A	2006-07	Medial	Upwards	3.03	-177.75	0.986
Pinot noir A	2006-07	Medial	Upwards	2.08	-120.72	0.988
Pinot noir A	2006-07	Medial	Upwards	4.00	-286.06	0.963
Pinot noir A	2006-07	Medial	Upwards	3.55	-135.13	0.992
Pinot noir A	2006-07	Medial	Upwards	3.03	-188.90	0.974
Pinot noir A	2006-07	Medial	Downwards	5.47	-344.11	0.991
Pinot noir A	2006-07	Medial	Downwards	5.40	-335.88	0.996
Pinot noir A	2006-07	Medial	Downwards	4.27	-227.68	0.992
Pinot noir A	2006-07	Medial	Downwards	3.11	-133.67	0.985
Pinot noir A	2006-07	Basal	Upwards	3.64	-168.91	0.969
Pinot noir A	2006-07	Basal	Upwards	5.17	-299.92	0.971
Pinot noir A	2006-07	Basal	Upwards	4.19	-248.34	0.980
Pinot noir A	2006-07	Basal	Upwards	3.74	-185.60	0.979
Pinot noir A	2006-07	Basal	Upwards	2.51	-101.50	0.965
Pinot noir A	2006-07	Basal	Downwards	3.55	-203.19	0.995
Pinot noir A	2006-07	Basal	Downwards	3.13	47.41	0.969
Pinot noir A	2006-07	Basal	Downwards	5.52	-214.63	0.979
Pinot noir A	2006-07	Basal	Downwards	3.61	-102.10	0.970
Pinot noir B	2005-06	Distal	Upwards	4.61	24.42	0.979
Pinot noir B	2005-06	Distal	Upwards	5.13	117.01	0.968
Pinot noir B	2005-06	Distal	Upwards	5.82	-77.84	0.985
Pinot noir B	2005-06	Distal	Upwards	5.43	-54.71	0.985
Pinot noir B	2005-06	Distal	Upwards	3.57	46.80	0.946
Pinot noir B	2005-06	Distal	Upwards	5.42	35.18	0.981

Pinot noir B	2005-06	Distal	Downwards	5.98	-60.61	0.997
Pinot noir B	2005-06	Distal	Downwards	4.57	-7.41	0.980
Pinot noir B	2005-06	Distal	Downwards	5.63	-89.93	0.981
Pinot noir B	2005-06	Distal	Downwards	5.90	-189.15	0.996
Pinot noir B	2005-06	Distal	Downwards	4.43	-2.01	0.959
Pinot noir B	2005-06	Distal	Downwards	4.62	9.53	0.959
Pinot noir B	2005-06	Medial	Upwards	1.89	65.96	0.927
Pinot noir B	2005-06	Medial	Upwards	3.61	-20.21	0.977
Pinot noir B	2005-06	Medial	Upwards	1.69	15.99	0.961
Pinot noir B	2005-06	Medial	Upwards	5.42	12.07	0.979
Pinot noir B	2005-06	Medial	Upwards	1.83	-26.00	0.953
Pinot noir B	2005-06	Medial	Upwards	4.55	-210.50	0.995
Pinot noir B	2005-06	Medial	Downwards	3.21	124.48	0.958
Pinot noir B	2005-06	Medial	Downwards	4.36	-117.08	0.988
Pinot noir B	2005-06	Medial	Downwards	3.39	-67.74	0.996
Pinot noir B	2005-06	Medial	Downwards	3.05	69.34	0.959
Pinot noir B	2005-06	Medial	Downwards	3.91	-100.66	0.991
Pinot noir B	2005-06	Medial	Downwards	3.84	-61.97	0.973
Pinot noir B	2005-06	Basal	Upwards	2.41	20.78	0.964
Pinot noir B	2005-06	Basal	Upwards	3.09	-41.30	0.963
Pinot noir B	2005-06	Basal	Upwards	3.50	-93.90	0.981
Pinot noir B	2005-06	Basal	Upwards	5.11	-199.58	0.996
Pinot noir B	2005-06	Basal	Upwards	5.01	-177.60	0.999
Pinot noir B	2005-06	Basal	Upwards	6.07	-294.20	0.985
Pinot noir B	2005-06	Basal	Downwards	3.61	-58.96	0.998
Pinot noir B	2005-06	Basal	Downwards	7.58	-412.41	0.959
Pinot noir B	2005-06	Basal	Downwards	5.32	-130.22	0.957
Pinot noir B	2005-06	Basal	Downwards	4.98	-172.21	0.999
Pinot noir B	2005-06	Basal	Downwards	2.09	-33.02	0.964
Pinot noir B	2005-06	Basal	Downwards	5.90	-253.37	0.990
Pinot noir B	2006-07	Distal	Upwards	3.46	-67.29	0.942
Pinot noir B	2006-07	Distal	Upwards	3.07	-76.54	0.977
Pinot noir B	2006-07	Distal	Upwards	3.76	-122.51	0.957
Pinot noir B	2006-07	Distal	Upwards	3.39	-127.98	0.979
Pinot noir B	2006-07	Distal	Upwards	3.79	-166.92	0.970
Pinot noir B	2006-07	Distal	Upwards	2.57	-34.87	0.968
Pinot noir B	2006-07	Distal	Downwards	2.14	24.12	0.900
Pinot noir B	2006-07	Distal	Downwards	4.66	-208.05	0.988
Pinot noir B	2006-07	Distal	Downwards	3.72	-28.76	0.938
Pinot noir B	2006-07	Distal	Downwards	4.61	-45.13	0.961
Pinot noir B	2006-07	Distal	Downwards	1.66	51.57	0.921
Pinot noir B	2006-07	Distal	Downwards	3.71	-177.81	0.990
Pinot noir B	2006-07	Medial	Upwards	0.48	6.94	0.863
Pinot noir B	2006-07	Medial	Upwards	1.90	-19.92	0.922
Pinot noir B	2006-07	Medial	Upwards	3.20	-154.14	0.983
Pinot noir B	2006-07	Medial	Upwards	0.46	-43.28	0.918
Pinot noir B	2006-07	Medial	Upwards	0.85	-66.49	0.994
Pinot noir B	2006-07	Medial	Downwards	0.83	-23.23	0.994
Pinot noir B	2006-07	Medial	Downwards	0.35	42.01	0.971
Pinot noir B	2006-07	Medial	Downwards	0.81	-63.39	0.993
Pinot noir B	2006-07	Medial	Downwards	4.31	-133.40	0.968
Pinot noir B	2006-07	Medial	Downwards	1.55	-23.86	0.970
Pinot noir B	2006-07	Medial	Downwards	0.16	-2.10	0.891
Pinot noir B	2006-07	Basal	Upwards	2.21	-8.62	0.938
Pinot noir B	2006-07	Basal	Upwards	4.12	-138.33	0.976
Pinot noir B	2006-07	Basal	Upwards	2.34	-3.42	0.950
Pinot noir B	2006-07	Basal	Upwards	2.00	-103.70	0.992
Pinot noir B	2006-07	Basal	Upwards	5.15	-219.20	0.980

Pinot noir B	2006-07	Basal	Downwards	0.77	-31.20	0.923
Pinot noir B	2006-07	Basal	Downwards	0.53	-29.10	0.977
Pinot noir B	2006-07	Basal	Downwards	2.38	-70.94	0.949
Pinot noir B	2006-07	Basal	Downwards	0.94	-29.55	0.984
Pinot noir B	2006-07	Basal	Downwards	3.06	-161.63	0.939